Influence of the genetic variability and plasticity on the fitness of a common intertidal seaweed

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Abstract

The ability of species to adapt to environmental change forms an important part of their stability and resilience. Many organisms show large phenotypic variation across environmental gradients and both terrestrial plant and sessile marine species often exhibit very different ecotypes in different environments. This phenotypic variation can be driven by a combination of genetic and non-genetic processes but their relative importance may differ between sites. In seaweeds, the potential to express phenotypic plasticity appears common and is of ecological importance as it increases fitness by allowing a response within the organism's life-time. In contrast, much less is known about the factors that might cause local adaptation in seaweeds. The habitat-forming seaweed *Hormosira banksii* plays an important role in structuring intertidal communities in southern Australia and several ecotypes are recognised across its distributional range. These different ecotypes are thought to reflect phenotypic responses to variable environmental conditions. In this thesis, I examined patterns of morphological variation in *Hormosira* and assessed the extent to which those patterns reflect environmental or genetic effects via reciprocal transplant experiments and their correlation with molecular variation. There was substantial morphological variation in *Hormosira* across its geographic distribution in Tasmania, Australia. In particular north coast individuals had a distinct 'bushy' morphology, smaller vesicles and shorter fronds, compared to other regions and tidal regime was identified as the best predictor of morphological differences between these regions. Semi-diurnal tides at Tasmania's north coast create more frequent and greater temperature fluctuations for *Hormosira* which likely results in higher levels of thermal and desiccation stress. To test whether the local

environmental factors determine phenotypic variation, juvenile *Hormosira* recruits were reciprocally transplanted between sites on the north and east coasts and their phenotype assessed after 12 months. These transplantations showed that the site of origin strongly affected morphology in *Hormosira* individuals after 12 months with none of the transplanted *Hormosira* developing a similar overall morphology to local *Hormosira* at the recipient site. *Hormosira* from the eastern populations did change in some traits when transplanted to the north coast indicating that certain morphological traits are more plastic than others which potentially allows them to adjust their morphology to conditions on the more stressful north coast. In contrast, individuals originating from the north coast had no plastic response when transplanted to the east coast suggesting stronger genetic control on morphology in those populations or, that the northern phenotype is established early in development. Moreover, transplantation mostly resulted in reduced growth rates and local individuals usually showed higher growth rates compared to foreign individuals, indicative of local adaptation. A second reciprocal transplant experiment of *Hormosira* embryos showed that embryos originating from the north coast had greater survival and growth in their local north coast environment compared to the east coast environment, reinforcing the idea that the northern origin phenotype may be locally adapted to greater emersion stress at the north coast. Survival of embryos from eastern populations did not vary between the local and foreign habitat but growth was enhanced in the foreign environment, indicating a capacity to benefit under the novel conditions. The phenotypic variation among *Hormosira* populations in Tasmania appears decoupled from cytochrome oxidase 1 (CO1) sequence variation with most Tasmanian populations having a single haplotype. However, across all southeastern Australia, there was clear partitioning of populations into three major haplotype groups (western, central and eastern)

which likely reflects events and changes prior to and during the Last Glacial Maximum (approximately 25,000 years ago), and subsequent patterns of re-colonisation of *Hormosira* into Tasmania. Current boundaries and break-points between haplotypes correspond with biogeographical provinces in this region and are possibly maintained by the short distance dispersal of *Hormosira* and a combination of habitat discontinuity and complex oceanographic features conditions that decrease the probability of long distance dispersal. Overall, this study indicates that morphological variation in *Hormosira* is influenced by both genetic and environmental factors but their relative importance appears population-specific. In particular, limited plasticity shown by both juveniles and embryos originating from the north coast highlight the significance of fixed genetic effects in possibly allowing the north coast morph to adapt to the greater environmental fluctuations on that coast.

Contents

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Declaration of Originality

"This thesis contains no material which has been accepted for a degree or diploma by the University or any other institution, except by way of background information and duly acknowledged in the thesis, and to the best of my knowledge and belief no material previously published or written by another person except where due acknowledgement is made in the text of the thesis, nor does the thesis contain any material that infringes copyright."

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Acknowledgement of co-authored papers

Chapters 2-5 of this thesis are written in a format suitable for publication in a scientific journal. Co-authors are acknowledged at the beginning of each chapter. Included in this thesis is one published article (Chapter 2), one under revision (Chapter 5) and two (Chapter 2 and 3) in preparation to be submitted for publication.

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Abbreviations

ADM	Average daily maximum
AIC	Akaike Information Criterion
AMOVA	Analysis of molecular variance
ANOVA	Analysis of variance
ASD	Average squared distance
BSA	Bovine serum albumin
BP	Before present
cox 1	Cytochrome Oxidase Subunit 1
dbRDA	Distance-based redundancy analysis
DISTLM	Distance-based linear models
EAC	Eastern Australian Current
ENSO	El Niño Southern Oscillation
F_{ST}	Genetic distance among populations
Hd	Haplotype diversity
K1	Luni-solar diurnal component of tide
LGM	Last glacial maximum
M2	Principle lunar component of tide
MDS	Non-metric multidimensional scaling
O1	Principle lunar diurnal of tide
PCO	Principal coordinates analysis
PCR	Polymerase chain reaction
PERMANOVA	Permutational multivariate analysis of variance
PERMDISP	Permutational multivariate analysis of dispersions

RGR	Relative growth rates
SE	Standard error
SIMPER	Similarity percentage analysis
SST	Sea surface temperature
STC	Subtropical Convergence Zone
S2	Principle solar component of tide
TAS	Tasmania
UV	Ultra-violet
π	Nucleotide diversity

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Chapter 1

General introduction

Canopy-forming macroalgae are among the main contributors to productivity on temperate rocky shores where they provide complex three-dimensional habitat, define community structure and support diverse food webs (Steneck et al. 2002). Their high productivity (Mann 1973, Dayton 1985) gives macroalgal ecosystems an ecological importance comparable to that of coral reefs (Steneck et al. 2002, Steneck and Johnson 2013). Large brown algae (class Phaeophycea) from the order Fucales are among the most abundant canopy-forming algae on temperate rocky reefs (Bertness 1999, Christie et al. 2009, Shepherd and Edgar 2013) with a worldwide distribution of almost 600 species (Guiry and Guiry 2016). Fucoid algae occur across heterogeneous landscapes ranging from exposed coasts to sheltered embayments, including estuaries and rockpools (Steinberg and Kendrick 1999) and span large vertical gradients from the intertidal to 30 m depth (Schiel and Foster 2006). The dense forests they create (Clayton 1984) are usually dominant in temperate regions where the average ocean temperature is below 20˚C.

Australia's temperate shores extend for approximately 5500 km and contain a great number of endemic species (Bolton 1994, Kerswell 2006). Much of the southern coastline consists of rocky reef inhabited by algal forest in which fucoid species are a prominent feature (Shepherd and Edgar 2013, Bennett et al. 2015). The Australian temperate coastline is characterised by a diverse landscape with large gradients in physical conditions (Coleman and Ayre 2007) driving high biodiversity and endemism across its range (Kerswell 2006, Bennett et al. 2015). In particular, ocean temperature declines with increasing latitudes and influences seaweed distribution patterns and their biogeography (Waters et al. 2010, Wernberg et al. 2013). In addition, major boundary currents flow along Australia's eastern (i.e. Eastern Australian Current) and western coastline (i.e. Leeuwin Current, extends into the Zeehan Current) and shape this region's ecology as they enhance connectivity

between habitats via the dispersal of larvae and propagules (Coleman et al. 2011b, Wernberg et al. 2013).

The marine intertidal contains a mosaic of environmental variation (Helmuth and Hofmann 2001) and there is great heterogeneity in abiotic stressors over multiple scales that determine species' tolerance and distribution patterns (Dring and Brown 1982, Chapman 1995, Davison and Pearson 1996). Intertidal species live at the interface of marine and terrestrial environments where daily tidal cycles subject them to a variety of environmental stressors from both of these systems (Helmuth et al. 2006, 2011, Lathlean et al. 2014). Marine environmental factors such as ocean temperature, wave energy, light, and nutrients impose significant stress on intertidal species (Davison et al. 1993, Davison and Pearson 1996, Helmuth and Hofmann 2001). However, stressors associated with the terrestrial climate during low tide including air temperature, wind, humidity and UV- radiation are often thought to be especially important for intertidal species (Bell 1995, Helmuth 1999). In particular, the effects of temperature and desiccation on the physiological tolerances of intertidal seaweeds during emersion play a major role in determining their fitness and distribution (Bell 1995, Dethier et al. 2005, Williams and Dethier 2005). However, emersion experienced by populations can fluctuate across spatial scales due to differences in vertical height (e.g. zonation) (Roberson and Coyer 2004, Hays 2007) or habitat topology (e.g. open coast versus estuary) (Zardi et al. 2013) resulting in different levels of emersion stress within and among sites. Moreover, the intensity of emersion stress strongly depends on the timing and duration of aerial exposure. For example, stress increases when long daytime exposure coincides with high summer temperatures (e.g. Helmuth et al. 2002) and has significant ecological consequences for intertidal communities (Helmuth and Hofmann 2001). However, little is known about how magnitude and spatial

variability of emersion stress affect the performance of intertidal organisms (Helmuth et al. 2002) and studies focussing on the interaction between terrestrial climate and tidal dynamics are exceedingly rare for intertidal seaweeds.

Dense fucoid canopies can reduce the stress associated with emersion for understory species because multiple layers of packed fronds provide shade, reduce temperature and light, and protect from desiccation (Schonbeck and Norton 1978, 1979, Stengel and Dring 1997). As such, the fucoid canopy forms a sheltered environment for invertebrates and other algal species, but also protects recruits of their own species from emersion stress (Johnson and Brawley 1998, Schiel and Foster 2006). Consequently, canopy-forming intertidal algae facilitate stability in the intertidal microhabitat (Schiel 2004), although their sessile growth makes them highly dependent on the surrounding climatic conditions (Pearson et al. 2009). Given, they are exposed to marine and terrestrial climatic conditions, intertidal algae provide useful early warning signs for changes within marine ecosystems (Pearson et al. 2009).

The ability to adjust morphology in response to changes in the environment forms a key part of species' resilience (Hoffmann and Sgrò 2011). Many seaweeds show intraspecific morphological variation across their distributional range and as a result, different populations often express different phenotypes or ecotypes (Dudley 1996, Blanchette 1997). Phenotypic variation can be caused by genetic (i.e. adaptation) and non-genetic (i.e. plasticity) processes (Pigliucci 1996) but their relative contribution can vary across sites (Hays 2007) and affect the functionality and ecology of the alga (Wright et al. 2012). Experimental evidence for the relative importance of genetic and non-genetic processes driving morphology in seaweeds is mixed; a large body of research indicates that morphological variation occurs as a

plastic response to local environmental conditions (Jordan and Vadas 1972, Sideman and Mathieson 1985, Kalvas and Kautsky 1993, Ruuskanen and Bäck 1999, Fowler-Walker et al. 2005, Koehl et al. 2008), but other studies have shown that morphology is fixed and that genotypes are well adapted to the local environmental conditions (Kalvas and Kautsky 1998, Scott et al. 2001, Blanchette et al. 2002, Roberson and Coyer 2004).

Adaptation to selection pressures imposed by variation in the abiotic environment is widespread (Kawecki 2008, Schmidt et al. 2008, Billard et al. 2010). In habitats with spatially divergent selection regimes and restricted gene flow, adaptation can lead to reproductive isolation and ultimately result in rapid species divergence such as found in the genus *Fucus* throughout the north Atlantic (Coyer et al. 2002, 2003, 2006a, 2006b). The driving forces of natural selection often vary at local scales and may also result in differential adaptation of populations within the same species to local conditions, leading to locally adapted populations (Hereford 2009). In such populations divergent selection favours advantageous traits that lead to higher individual fitness in their native habitat compared to foreign phenotypes from different habitats (Kawecki and Ebert 2004). While local adaptation is an important mechanism maintaining genetic variability (Kawecki and Ebert 2004) and heritable trait selection (Monro et al. 2007) between populations, it can also limit the evolvability within populations due to the reduced influence selective pressures have on such specialist genotypes (Eads et al. 2012). Moreover, selection of traits can vary with different environmental regimes and may not always result in adaptation and consequently different populations may have different capacities to respond to change (Hoffmann and Merilä 1999). It is thus important to understand the drivers of natural selection to be able to determine the evolutionary potential

and structure of populations living under various environmental conditions (Hoffmann and Merilä 1999).

Under a changing environment, the persistence of a population either depends on the stress tolerance of particular genotypes or a high ability to express phenotypic plasticity (Volis et al. 1998). Transplant experiments help to explore patterns of local adaptation and assess species' evolutionary potential in different environments. However, evidence for local adaptation largely come from studies of terrestrial plants which often have restricted dispersal and limited gene flow (Leimu and Fischer 2008). In contrast, marine populations are usually open and have long been thought to have limited potential for local adaptation due to long-distance dispersal and extensive gene flow (Sanford and Kelly 2011). Nevertheless, an increasing body of research demonstrates that the scales of dispersal and connectivity in marine species are often smaller than expected and that local adaptation is more common than previously thought (Palumbi 2004, Sanford and Kelly 2011). In particular organisms with direct-developing life histories and limited dispersal show increased potential for local adaptation to particular environmental regimes (Bohonak 1999).

Some of the most compelling evidence for local adaptation in seaweeds has come from fucoid algae, presumably because of the harsh selection regimes these species often live in (e.g. in response to varying gradients in salinity: Serrão et al. 1996a, Pearson et al. 2000; emersion time: Zardi et al. 2011, 2013; and latitude: Araújo et al. 2011). Moreover, transplant experiments with the fucoid *Silvetia compressa* showed adaptive differentiation between populations across a zonal gradient and thus linked local adaptation with small scale variation in emersion (Hays 2007). Fucoids can be monoecious or dioecious, fertilisation usually occurs externally and they have

a direct-developing life history with no free-living gametophyte phase (Schiel and Foster 2006). After release, female gametes sink close to their parental stand and attract sperm via pheromones, a mechanism that works efficiently only over very short distances (µm – mm) (Müller et al. 1985, Serrão et al. 1996b). Generally, fucoid reproduction is often tightly cued to periods of calm water within the tidal cycle and fertilisation success can be reduced under turbulent water conditions (Serrão et al. 1996b, Taylor and Schiel 2003). Moreover, while detached thalli are often capable of floating and theoretically can disperse via rafting, this appears to contribute little to gene flow in dioecious fucoids (Coyer et al. 2003, McKenzie and Bellgrove 2008; but see Coleman and Kelaher 2009). Consequently, this low capacity for dispersal indicates a high potential for locally adapted populations and thus makes representatives of this group the perfect candidates for investigating the influence of environmental variability on phenotypic and genotypic performance.

The monotypic, canopy forming fucoid *Hormosira banksii* (Turner) Descaisne (hereafter referred to as *Hormosira*) creates complex habitat structures in the intertidal (Womersley 1967, Underwood 1999). It is widespread and very abundant occurring from sheltered to moderately exposed shores in the temperate regions of Australia, New Zealand and nearby islands (Womersley 1967). As the most common intertidal fucoid in these regions it has been the subject of multiple studies highlighting its influence as a foundation species on communities (Keough and Quinn 1998, Underwood 1999, Bishop et al. 2009, Hughes et al. 2014). *Hormosira* beds show slow recovery from loss or disturbance (Underwood 1998, Schiel 2006) which affects the diversity and structure of the understorey community (Lilley and Schiel 2006, Schiel and Lilley 2011, Tait and Schiel 2011). However, across its distributional range *Hormosira* occurs in a range of different environments, shows large morphological variation and several ecotypes are recognised (Osborn 1948,

Bergquist 1959, Clarke and Womersley 1981, King 1981, Ralph et al. 1998, Macinnis-Ng et al. 2005, Bishop et al. 2009). While phenotypic variation in *Hormosira* has been assumed to be plastic and induced by environmental conditions in each habitat (e.g. Osborn 1948, Womersley 1967, Ralph et al. 1998, Macinnis-Ng et al. 2005), the actual drivers of morphological variation in *Hormosira* remain unclear as no studies have used long-term transplant experiments to determine whether *Hormosira* ecotypes reflect plastic responses or genotypes adapted to local environmental conditions.

The overall aim of this thesis is to characterise patterns of morphological and genetic variation in *Hormosira* across multiple spatial scales to identify the potential underlying drivers of this variation, and to determine the extent to which phenotypic and adaptive responses contribute to fitness in this important habitat-forming seaweed.

The specific objectives of the four data chapters in this thesis are to:

- Describe morphological variation in *Hormosira* from open coast habitats in Tasmania, Australia at multiple spatial scales, quantify variation in relevant environmental variables at those sites and link morphological patterns with environmental variation (Chapter 2)

- Determine to what extent the environment interacts with the morphology and growth of *Hormosira* and test whether different ecotypes from Tasmania's north and east coast reflect plastic or fixed traits (Chapter 3)

- Explore patterns of local adaptation in *Hormosira* embryos from habitats with different environmental conditions, and test whether survivorship and

performance decline when embryos are transplanted outside their local environments (Chapter 4)

- Examine patterns of genetic structure and diversity of *Hormosira* populations in southeastern Australia using the Cytochrome Oxidase 1 region of the mitochondrial genome, and assess the roles of palaeohistoric and contemporary factors in determining genetic divergence patterns in *Hormosira* in southeastern Australia (Chapter 5)

Chapter 2

Environmental correlates of phenotypic variation: do variable tidal regimes influence morphology in intertidal seaweeds?

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Introduction

Habitat-forming seaweeds play an important role in temperate marine ecosystems because they create structurally complex habitats and support diverse and productive food webs (Steneck et al. 2002, Graham et al. 2007). All seaweeds experience environmental variation across their distributional ranges and different populations often have different morphologies (Blanchette 1997, Kalvas and Kautsky 1998, Wernberg and Vanderklift 2010). The underlying mechanisms for this morphological variation can be genetic (Hays 2007) or environmental (Fowler-Walker et al. 2005, Koehl et al. 2008, Miller et al. 2011). In particular, wave exposure affects the morphology of kelp (Blanchette et al. 2002, Fowler-Walker et al. 2005, Wernberg and Vanderklift 2010) and fucoids (Kalvas and Kautsky 1993, 1998, Blanchette 1997). Typically, increasing wave exposure is correlated with a smaller thallus and vesicle size (Bergquist 1959, Ruuskanen and Bäck 1999, Blanchette et al. 2002, Thomsen et al. 2004) although an increase in thallus size with increasing wave exposure has been described (Cheshire and Hallam 1988, Bekkby et al. 2014).

Other environmental factors can affect seaweed morphology including sea surface temperature and nutrients (Mabin et al. 2013), light regime (Lüning 1992, Wing et al. 2007) and currents (Kalvas and Kautsky 1998). For intertidal seaweeds, exposure at low tide results in daily fluctuations in a range of important environmental parameters (temperature, humidity, salinity, resource supply and light) (Davison and Pearson 1996, Billard et al. 2010) which have important implications for photosynthetic rates (Williams and Dethier 2005), growth (Schonbeck and Norton 1980, Wright et al. 2004) and abundance (Chapman 1995). Thus, tidal variation within (i.e. different zones) and among (i.e. different tidal regimes) sites will influence the time intertidal seaweeds are exposed to the air and those harsh abiotic

conditions. A number of studies have shown that intertidal seaweeds have lower growth and reproduction in high intertidal zones, where exposure at low tide is longest and thermal and desiccation stress are greatest, compared to individuals in lower intertidal zones (e.g. Hawkins & Hartnoll 1985; Stengel & Dring 1997; Ramage & Schiel 1998) although this can vary with season if the timing of the low tide changes (Wright et al. 2004). Patterns of tidal exposure are determined by the dominant tidal regime (semi-diurnal, mixed, or diurnal) but little is known about how seaweed morphology changes with variations in the tidal regime.

The temperate, intertidal brown macroalga *Hormosira banksii* (Turner) Descaisne (Fucales, Phaeophyceae) is very abundant in southern Australia and New Zealand (Womersley 1967) and plays an important role in structuring intertidal communities (Povey and Keough 1991, Keough and Quinn 1998, Schiel 2004). Environmental conditions influence the morphology of *Hormosira* and larger plants with larger vesicles generally occurring in estuaries and sheltered rockpools as opposed to exposed coastal shores (Macinnis-Ng et al. 2005). Lower wave exposure in these sheltered areas is suggested as the mechanism affecting the vesicle size of *Hormosira* (Bergquist 1959, Macinnis-Ng et al. 2005) although there is no experimental evidence to support this suggestion (Ralph et al. 1998). Most studies of seaweed morphology consider the role of a single environmental parameter in isolation (e. g. wave action/exposure: Jackelman & Bolton 1990; Ruuskanen et al. 1999; Wernberg & Thomsen 2005; Fowler-Walker et al. 2005; light: Monro & Poore 2005; temperature: Höffle et al. 2011). To better understand the patterns of seaweed morphology, the relative contribution of multiple environmental factors needs to be investigated (Santos 1993, Lawton 1996, Dethier and Williams 2009). In addition, studies rarely incorporate multiple spatial scales (including zones for intertidal species) reflecting variation in environmental factors (although see

Williams & Dethier 2005; Coleman & Muhlin 2008). Generalising from studies at small spatial-scales (several meters) is difficult and large-scale studies are needed to explain the extent to which small-scale patterns can be generalised to larger scales (Lawton 1996, Wootton 2001, Terlizzi et al. 2007) and the influence that regional processes have on local populations (Fraschetti et al. 2005). Diverse abiotic factors potentially affecting seaweed morphology vary across spatial scales and emphasise the need for a multi-scale sampling design.

Here we examine the relationships between the morphology of *Hormosira banksii* and environmental factors across multiple spatial scales around the island of Tasmania, Australia. Tasmania is situated south of mainland Australia and its different coasts are impacted by different currents, variable wave activity and different tidal regimes. The dominant current on the east coast is the Eastern Australian Current (EAC) and the dominant current on the west coast is the Zeehan Current (ZC). Both transport warm, nutrient poor water southwards (Baines et al. 1983, Ridgway 2007) but are strongest at different times of the year: the EAC reaches maximum flow during summer and typically reaches the tip of south Tasmania while the ZC is strongest in winter and extends around south Tasmania (Harris et al. 1987, Ridgway 2007). Wave activity is strong on the south, west and east coasts of Tasmania while the north coast has higher tides than the other coasts (Short 2006a). We focussed on *Hormosira* on open rocky platforms at similar tidal heights at multiple sites to determine 1) variation in the morphology of *Hormosira* among regions (100s km apart), sites within regions (10s km apart) and zones within sites (metres apart), 2) variation in relevant environmental factors (wave, tidal and temperature parameters) at those sites, and 3) the link between morphological variation and environmental factors.

Material and methods

Study sites and sampling

Hormosira banksii was sampled in four regions around Tasmania between January and March 2013. These four regions were the north, northeast, southeast and west coasts of Tasmania (Figure 2.1) and reflected four bioregions (Boags, Freycinet, Bruny and Franklin respectively) as designated by the Integrated Marine and Coastal Regionalisation of Australia (IMCRA 2006). We did not sample the southwest coast as this region is very isolated and largely inaccessible. In each of the four regions we collected individuals from 3 sites that were at least 25 km apart (Figure 2.1). At every site 30 individuals were collected from each of the eulittoral and sublittoral to incorporate possible small-scale changes in morphology between intertidal zones (individuals that were completely submerged during the lower low tide were considered as sublittoral). We sampled whole individuals (one to several fronds arising from the same holdfast) instead of collecting one frond per individual (see Ralph et al. 1998; Macinnis-Ng et al. 2005) to take the overall morphology of individuals into account. Morphological variables were either measured immediately at the site or back in the lab depending on the remoteness of the site. Individuals measured back in the lab were transported in seawater filled Ziploc-bags placed in coolers and were always measured within 12 hours of collection.

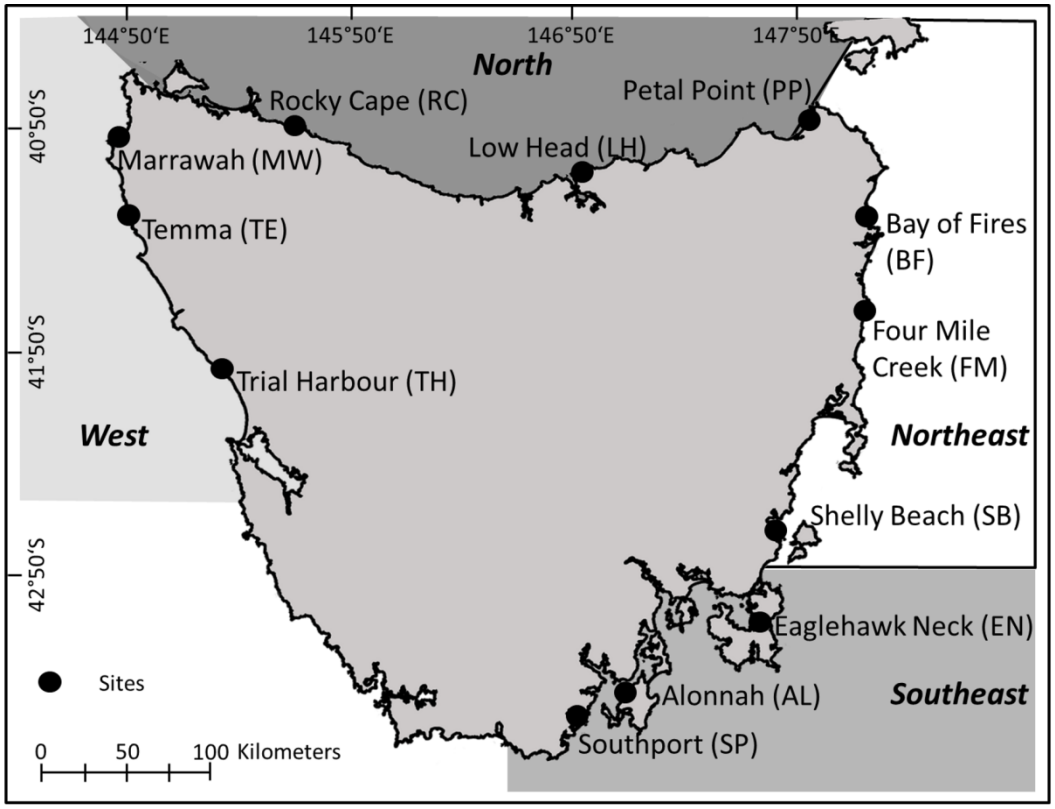

Figure 2.1: Map of Tasmania showing the three sites (with abbreviations) sampled within each of the four regions.

Morphological variation

In *Hormosira*, one to several fronds arise from the holdfast and can grow to 300 mm in length (Edgar 1997). Thalli have apical growth (Clayton 1984) and are comprised of chains of water-filled vesicles of variable morphology that are joined by 'connectives' (Osborn 1948, Bergquist 1959, Clarke and Womersley 1981, Macinnis-Ng et al. 2005). Often more than one connective grows from the lower vesicle resulting in different forms of branching (i.e. branching occurs if two or more connectives grow from the lower vesicle). Seven morphological traits that reflect the overall size and shape of *Hormosira* thalli were measured. These were: total length (the length of the longest frond), branching order (the highest number of branching points available on an individual, branching order ranged from 2 to 8), branching

structure (the highest number of connectives coming from a vesicle), total number of vesicles per individual and three vesicle traits (vesicle length, widest width and cell wall thickness). Vesicle traits were measured on ten healthy, non-branched vesicles that were randomly selected from the longest frond of each individual (for standardisation we selected 3 vesicles from the basal third of the thallus, 4 from the mid third of the thallus and 3 from the apical third of the thallus). Vesicle cell wall thickness was measured from the middle position of sectioned vesicles. Mean values for the three vesicles traits were then calculated for each individual.

Environmental variation

We extracted or calculated thirteen environmental variables for each site that reflected variation in wave exposure, tidal regimes, sea surface temperature and air temperature (Table S1 in the Supporting Information). The wave exposure at each site was calculated using the Baardseth Index (Baardseth 1970), a commonly used cartographic method to quantify exposure (Ruuskanen et al. 1999, Wernberg and Thomsen 2005, Wernberg and Vanderklift 2010). This index considers wave effects from multiple directions and correlates well with wave height and maximum water velocity (Wernberg and Vanderklift 2010). A nautical chart (1:33333 scale) was used to calculate the Baardseth Index by dividing adjacencies of each site into 40 sectors of an angle of 9˚. Sectors were excluded when skerries, islands, mainland shore or other obstacles were present within a fetch of 7.5 km. Thus, the resulting index refers to the sum of free sectors where 0 represents complete shelter and 40 represents absolute exposure. The maximum wave height and the mean spring and neap tides for each site were extracted from the literature (Short 2006a). This tidal data was then used to calculate the maximum tidal difference (i. e. mean spring tide minus mean neep tide) at each site. We also determined the tidal regime for each site as semi-diurnal, mixed, mainly diurnal and diurnal. Approximately 70% of a

tide's total period, form and amplitude can be mainly explained by four components: 12.42 h principle lunar (M2), 12.0 h principle solar (S2), 23.93 h luni-solar diurnal (K1) and 25.82 h principle lunar diurnal (O1) (MacMillan 1966, Barnwell 1976, Thurman 2004). The contribution of each of those constituents can vary among sites, resulting in sites that differ with respect to their tidal environments (Thurman 2004). Based on the following ratio of these tidal components the tidal regime was calculated for each site:

$$\frac{K1 + O1}{M2 + S2}$$

Sites with a tidal regime ratio of 0.25 or less are classified as semi-diurnal, sites with a ratio between 0.25 and 1.5 are classified as mixed, mainly semi-diurnal and sites with a ratio from 1.5 to 3.0 are classified as mixed, mainly diurnal. A ratio of 3.0 or greater describes sites with diurnal tides (Thurman 2004).

Daily minimum and maximum mean air temperature data for 2011 were obtained from the Bureau of Meteorology (Bureau of Meteorology 2011) using the weather station closest to each site. Minimum and maximum annual mean, summer mean and winter mean temperatures were then calculated. Sea surface temperature (SST) was derived over the period from 1997 – 2009 (from satellite imagery) with mean values calculated for each site. In this study, we used AVHRR Pathfinder Version 5.2 (PFV5.2) data, obtained from the US National Oceanographic Data Center and GHRSST (http://pathfinder.nodc.noaa.gov). The PFV5.2 data are an updated version of the Pathfinder Version 5.0 and 5.1 collection described in Casey et al. (2010). Global daily imagery was downloaded and averaged across the period from 1997 to 2009 and then sub-scened to the study area. A 13-year SST average for each study site was extracted for each of the sampling sites. Annual averages, also generated

with this data, encompass inter-annual variation in SST, taking into account warm and cool water phases associated with the El Niño Southern Oscillation (ENSO) and reflect the variable sea surface temperature environment for *Hormosira* at these sites.

Statistical analyses

We used permutational multivariate analysis of variance (PERMANOVA; Anderson 2001) to test for differences in morphology between region (fixed), site (random) nested within region and zone (fixed). Data were 4th root transformed (to reduce the impact of quantitatively dominant values on the analysis) and normalised before Euclidean distances were calculated. Permutations (N = 9999) were applied to residuals under the full model for PERMANOVA. Post-hoc pairwise comparisons were then used to explore significant factor effects using 9999 permutations. No corrections for the inflation of type I error rates were applied as p-values obtained by permutation test each null hypothesis individually (Anderson et al. 2008). Monte-Carlo p-values were used for pairwise comparisons when low numbers of unique permutations (i. e. < 100) were obtained as their test statistic is more accurate when there are not enough possible permutations (Anderson et al. 2008). Effect sizes were calculated for each factor as the percentage of the square rooted estimated components of variation (Anderson et al. 2008). In cases where negative estimates of components of variation were obtained, these terms were consecutively pooled (Anderson et al. 2008). We tested for differences in multivariate dispersion between regions and zones (significant fixed factors from PERMANOVA, see results) using the PERMDSIP routine (Anderson et al. 2008), to examine whether some populations were morphologically more variable than others. Using the ANOVA F statistic PERMDISP compares distances between observations and their group centroid (Anderson et al. 2008). Results obtained via PERMANOVA are considered to be

robust to some heterogeneity in dispersion (Anderson et al. 2008). Non-metric multidimensional scaling (nMDS) was used to visualise the multivariate differences in morphology between sites and zones. Ordinations were based on calculated centroids for zones at each site to reduce the observation numbers and focus on the distance between zones within sites and between sites. Similarity percentage (SIMPER) was used to test for the relative contribution of each morphological trait to the differences between regions (Clarke 1993). In this procedure the average between-group dissimilarities (average squared distance, ASD) were fragmented into individual contributions from each morphological variable. We also used principal coordinates analysis (PCO) to project actual dissimilarities between individual thalli to visualise distances between individuals across regions, sites and zones.

Morphological traits were analysed individually with ANOVA using the same model as above. The PERMANOVA routine was used here as the null distribution of the test statistic is achieved via permutation which avoided violation of ANOVA assumptions and it is possible to interpret interaction terms involving random factors (Anderson et al. 2008). With a single response variable on a Euclidean distance matrix, the resulting F ratio is the same as in the traditional ANOVA (Anderson et al. 2008). Posthoc pairwise comparisons were done with significant factor effects.

The distribution of the environmental data was visualised via a Draftsman plot to check for interrelations between variables. Spring tide and neap tide were square root transformed as they were mildly right skewed. Highly correlated air temperature variables were removed. Summer mean maximum ($r = 0.99$) and winter mean maximum ($r = 0.98$) were both highly correlated with annual mean maximum temperature. Likewise summer mean minimum ($r = 0.90$) and winter

mean minimum (r = 0.97) were strongly correlated with annual mean minimum temperature. Subsequently, only annual mean maximum and minimum temperatures were used. The remaining nine variables were normalised and patterns of environmental variation among sites visualised using PCO. Distance-based linear models (DISTLM) were used to link the morphological data with the environmental variables (Anderson et al. 2008). Based on multivariate regression, DISTLM models the relationship between the multivariate morphological data cloud and the environmental predictor variables. To find the best possible model for a number of given variables and thus, the most parsimonious model the routine followed the AIC model (An Information Criterion, Akaike 1973) selection criteria and Best as a selection procedure. Smaller values of the AIC indicate a better model (Anderson et al. 2008) and yield into the 'best' combination of environmental predictor variables that explain the largest amount of variation in the morphological response variables. Marginal tests further explain the percentage variance when each single environmental variable is considered individually together with a 'Pseudo-F statistic'. The relationship between the morphological and environmental data was visualised (based on Pearson's correlation) via a distance-based redundancy analysis (dbRDA). The dbRDA routine performs a constrained ordination of the morphological data using the DISTLM model. The axes in the dbRDA were constrained to the most parsimonious model (identified by the DISTLM routine for each number of variables) to explain the variation in morphology with a smaller set of predictor variables in order to identify environmental variables that have the strongest impact on *Hormosira* morphology.

Results

Morphological variation

The morphology of *Hormosira* varied across all spatial scales (Table 2.1). Differences among regions accounted for 28.4 % of the total variation in morphology and pairwise comparisons showed significant differences between the north and each of the other regions (west: $p < 0.05$; northeast: $p < 0.05$; southeast: $p < 0.01$) but no other regions differed from each other. Differences among sites within regions accounted for the 25.46 % of total variation (Table 2.1). The pooled interaction term (region x zone was pooled with site [region] x zone due to its negative estimates of components of variation) was also significant and highlighted the small-scale variation within sites. Post-hoc comparisons showed significant differences in *Hormosira* morphology between the eulittoral and sublittoral zones at all sites except for Four Mile Creek in the northeast and Alonnah in the southeast (both $p > 0.05$). Comparisons for the same zone across different sites within each region also showed significant results for all combinations ($p < 0.01$). Thus, not only are there regional patterns of morphological variation in *Hormosira*, but there is significant small-scale variation among zones and sites. Variation among individuals within zones at sites accounted for 29.65 % of the total morphological variation (Table 2.1).

Table 2.1: PERMANOVA testing the effects of Region, Site within Region and Zone on *Hormosira* morphology. SS and degrees of freedom for the term Region x Zone were pooled with the term Site [Region] x Zone due to the negative estimates of components of variation (Graham and Edwards 2001). Effect represents the percentage that each factor contributes to the components of variation.

Source	**df**	**MS**	**Pseudo-F**	**Effect (%)**	**pairwise comparisons**		
Region	3	602.06	**4.65 *****	28.39	N ≠ NE; N ≠ SE; N ≠ W		
Zone	1	66.93	**5.60 ****	6.85			
Site [Region]	8	129.52	**45.25 *****	25.46			
Pooled	11	11.96	**4.18 *****	9.65	Eu ≠ Sub for	TH, TE, MW	W
						RC, LH, PP	N
						BF, SB	NE
						EN, SP	SE
Residuals	696	2.86		29.65			

Bold text refers to significant factor effects (asterisks represent the significance level: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$). Pairwise tests show Regions or Zones (within each site) that differed ($p < 0.05$). Non-significant pairwise tests are not shown. Site abbreviations as in Table 2.1; Abbreviations for posthoc tests: N = North, NE = Northeast, SE = Southeast, W = West; Eu = eulittoral zone, Sub = sublittoral zone. Abbreviations for sites as in Figure 2.1.

Morphological dispersion (heterogeneity) of samples depended on the region where *Hormosira* was collected. Significant differences were observed between all regions ($p < 0.001$) except between the northeast and southeast regions. Individuals from the north region showed greater variability and a higher multivariate dispersion (mean multivariate dispersion = 2.37) compared to other regions and their centroids were dispersed most in the nMDS (Figure 2.2). Sites in the north region (especially Low Head and Petal Point) separated from each other and were distinct from sites in other regions (Figure 2.2). Individuals along the east coast showed similar dispersion (mean multivariate dispersion northeast = 1.73; mean multivariate dispersion southeast = 1.74) and both had lower dispersion than the

north and west regions (mean multivariate dispersion west = 2.01). PERMDISP also revealed a significant increase ($p < 0.01$) in dispersion among samples in the sublittoral habitat (mean multivariate dispersion = 2.55) compared to the eulittoral (mean multivariate dispersion = 2.33). Site centroids for sublittoral samples appeared to be most distant in the nMDS and occupied the outer margins in the plot (Figure 2.2). Western and eastern sites formed distinct geographical groupings in the nMDS (Figure 2.2). Conversely, the nMDS suggests more similar morphology between two sites belonging to different regions (Trial Harbour and Alonnah) than to sites of the same region. Variation in morphology between zones occurs at northern and western sites (except for Rocky Cape) whereas individuals from the east coast had a similar morphology across eulittoral and sublittoral zones (Figure 2.2).

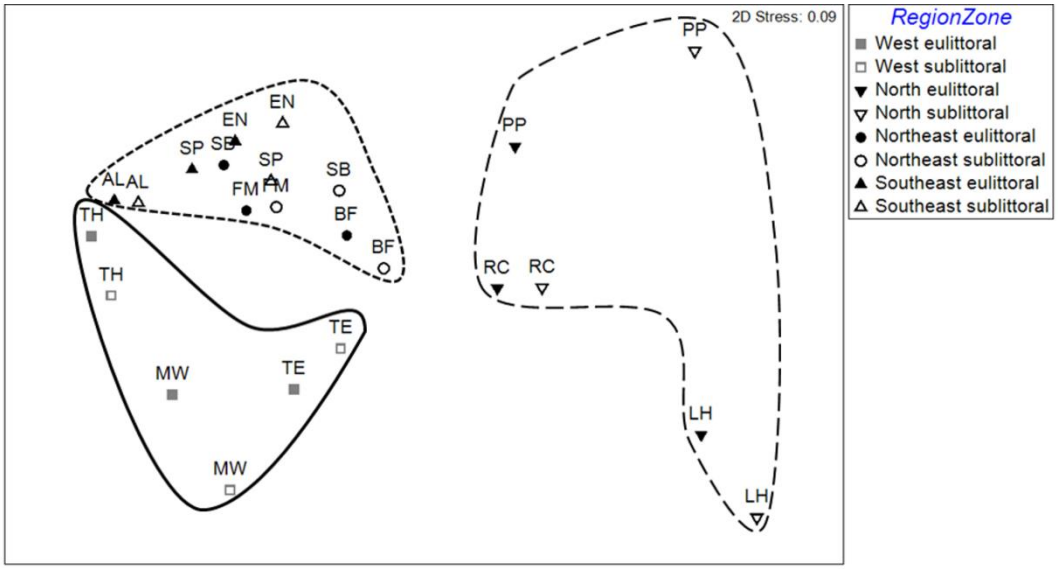

Figure 2.2: nMDS of *Hormosira* morphology based on centroids calculated for each zone at each site. West region sites are enclosed by the continuous black line, north region sites are enclosed by the dashed line and east coast sites (northeast and southeast region) are enclosed by the dotted line. Site abbreviations as in Figure 2.1.

PCO 1 suggested similar gross morphology for *Hormosira* in the southeast, west and northeast which was distinct from *Hormosira* in the north (Figure 2.3). Vectors for morphological traits indicated strong negative loadings along PCO 1 for vesicle length (r = -0.899) and width (r = -0.865, i. e. shorter, narrower vesicles in north region) and strong positive loadings for total vesicle number per thallus (r = 0.653, i. e. more vesicles in the north region). PCO 2 identified a relatively large spread in morphology among individuals form the north region (Figure 2.3). Vectors for maximum thallus length (r = -0.697) and branching order (r = -0.697) both had strong negative loadings on PCO 2 but did not identify any separation among regions along that axis.

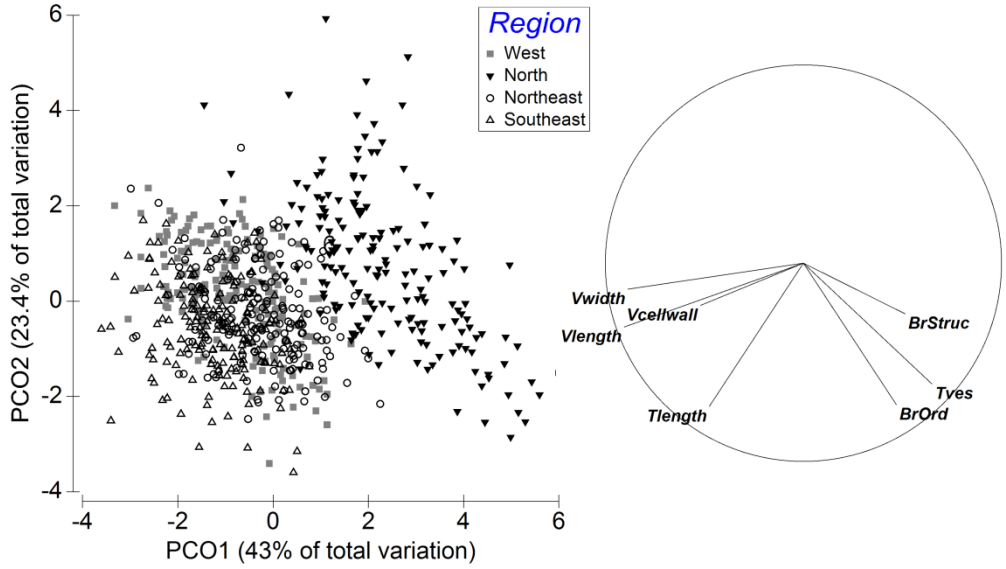

Figure 2.3: PCO ordination of *Hormosira* morphology. Each individual is represented by a symbol corresponding to one of the four regions. PCO1 and PCO2 refer to the first and second principal coordinates axes and indicate the percentage of variation explained by each axis. Inset shows the vectors based on Pearson correlation for the morphological traits (Vlength: vesicle length, Vwidth: vesicle width, Vcellwall: vesicle cell wall, Tlength: total length, Tves: total number of vesicles, BrOrd: branching order, BrStruc: branching structure).

SIMPER analysis revealed a relatively mixed contribution of morphological variables to the differences found between regions and zones. No single morphological trait or clear pattern could be described as strongly contributing to any of the comparisons (Table 2.2). Nevertheless, the highest average squared distance (ASD) occurred between the north region vs. all other regions and the lowest ASD occurred between the northeast and southeast regions. In most comparisons, vesicle traits appeared to be more important in explaining differences between regions: vesicles length always strongly contributed to differences between the north region and other regions while vesicle cell wall thickness was the highest contributor to differences between the west region vs. other regions (Table 2.2).

Table 2.2: SIMPER analyses showing the relative contribution (%) of each morphological trait in *Hormosira* to multivariate differences between regions. ASD = Average Squared distance. Abbreviations for morphological traits: Vlength = vesicle length, Vwidth = vesicle width, Vcellwall = vesicle cell wall, Tlength = total length, Tves: total number of vesicles, BrOrd: branching order, BrStruc: branching structure.

	North & West		**North & Northeast**		**North & Southeast**		**West & Northeast**		**West & Southeast**		**Northeast & Southeast**	
	ASD = 22.31		**ASD = 18.11**		**ASD = 24.66**		**ASD = 10.71**		**ASD = 10.66**		**ASD = 7.87**	
Rank	Variable	%	Variable	%	Variable	%	Variable	%	Variable	%	Variable	%
1	Vcellwall	22.34	Vlength	19.7	Vlength	23.05	Vcellwall	29.64	Vcellwall	21.05	BrOrd	20.46
2	Vlength	19.72	Tlength	17.48	Vwidth	18.52	BrOrd	17.61	Tlength	20.77	Tves	16.03
3	Tves	13.22	BrStruc	15.75	Tlength	17.59	Tlength	14.34	BrOrd	16.65	BrStruc	14.84
4	BrStruc	12.99	Tves	14.54	BrStruc	13.38	Tves	12.93	Vwidth	15.65	Vwidth	14.67
5	Vwidth	12.54	Vwidth	14.46	Tves	11.98	Vwidth	10.82	Tves	10.82	Tlength	13.29
6	BrOrd	11.83	BrOrd	12.87	BrOrd	8.23	BrStruc	10.09	BrStruc	10.6	Vcellwall	12.75
7	Tlength	7.36	Vcellwall	5.2	Vcellwall	7.26	Vlength	4.55	Vlength	4.45	Vlength	7.96

Analyses of the individual morphological traits showed that vesicle length, vesicle width, vesicle cell wall thickness and the total length of the longest thallus varied among regions (Table 2.3). Overall, these differences reflected the generally smaller size of *Hormosira* in the north region compared to other regions: individuals from the north region were smaller for vesicle length, vesicle width and total length of the longest thallus compared to the northeast region; smaller for vesicle length, vesicle width and thallus length compared to the southeast region and smaller for vesicle length and vesicle cell wall thickness compared to the west region (Table 2.3, Figure 2.4). Northeast region individuals had thinner vesicle cell walls than west region individuals (Table 2.3, Figure 2.4). Thalli of west region individuals were shorter compared to southeast region individuals (Table 2.3, Figure 2.4). Total number of vesicles, branching order and branching structure did not differ among regions. All morphological traits except branching structure showed significant small-scale variation (i.e. significant site [region] x zone interaction (Table 2.3, Figure 2.4). Where differences occurred between zones within sites, generally, vesicle traits (length, width and cell wall thickness) were larger in the eulittoral zone. In contrast, thallus traits (number of vesicles, thallus length and branching order) were more variable: greater values for these traits occurred in the eulittoral or subittoral zones at different sites (Table 2.3, Figure 2.4).

Table 2.3: ANOVA testing the effect of region, site within region and zone on individual morphological traits of *Hormosira*. Analyses were based on Euclidean distances on 4th root transformed data following 9999 permutations of residuals under the full model (Anderson et al. 2008). Effect represents the relative contribution of each factor to the components of variation when negative components of variation were set to 0 (Graham and Edwards 2001). Pairwise posthoc comparisons were done on significant terms for region and site[region] x zone.

Source	**Df**	**MS**	**F**	**Effect (%)**	**Posthoc comparisons**	**MS**	**F**	**Effect (%)**	**Posthoc comparisons**
		Vesicle length				*Vesicle width*			
Transformation		Fourth-root				log (x)			
Re	3	4.21	**26.66** ***	50.04	N < NE, N < W, N < SE	0.44	**5.29** *	31.54	N < NE, N < SE
Zo	1	0.22	**8.95** *	7.78		0.13	**22.64** **	13.38	
Si(Re)	8	0.16	**67.77** ***	16.99		0.08	**109.33** ***	26.25	
RexZo	3	0.00	0.92	0.0		0.00	0.03	0.0	
Si(Re)xZo	8	0.02	**10.57** ***	9.09	Eu > Sub: MW, TH, PP, SB, EN, SP	0.01	**7.81** ***	9.30	Eu > Sub: MW, TH, RC, LH, PP, BF, SB, EN, SP
Res	696	0.00		16.10		0.00		19.53	
		Vesicle cell wall				*Total length*			
Transformation		Fourth-root				log (x)			
Re	3	0.36	**8.22** **	36.22	N < W, NE < W	0.78	**9.98** **	35.49	N< NE, N < SE, W < SE
Zo	1	0.02	4.13	5.97		0.00	0.15	0.00	
Si(Re)	8	0.04	**57.63** ***	23.14		0.08	**31.67** ***	20.18	
RexZo	3	0.00	0.28	0.0		0.01	0.46	0.0	
Si(Re)xZo	8	0.01	**7.23** ***	10.85	Eu > Sub: TE, LH, PP, SB, SP; Sub > Eu: MW	0.03	**10.77** ***	16.12	Eu > Sub: TH, PP, SB Sub > Eu: TE, LH, BF
Res	696	0.00		23.82		0.00		28.23	
		Total vesicles				*Branching Order*			
Transformation		Fourth-root				Fourth-root			
Re	3	11.60	0.43	35.74		0.23	0.83	0.00	
Zo	1	3.09	2.74	0.00		0.01	0.25	0.00	
Si(Re)	8	26.85	**96.5** ***	19.82		0.27	**25.92** ***	32.46	
RexZo	3	0.06	0.05	0.0		0.03	0.6	0.0	
Si(Re)xZo	8	1.13	**4.05** ***	15.54	Eu > Sub: PP; Sub > Eu: TE, LH, BF, SP	0.05	**4.49** ***	17.17	Eu > Sub: PP Sub > Eu: LH, BF
Res	696	0.28		28.90		0.01		50.37	
		Branching Structure							
Transformation		log (x)							
Re	3	0.07	1.814	13.25					
Zo	1	0.02	**5.5** *	6.9					
Si(Re)	8	0.04	**16.76** ***	24.67					
RexZo	3	0.00	0.87	0.0					
Si(Re)xZo	8	0.00	1.64	7.05					
Res	696	0.00		48.13					

Bold text refers to significant factor effects (asterisks represent the significance level: *** p < 0.001, ** p < 0.01, * p < 0.05). Abbreviations for sites as in Figure 2.1. Abbreviations for Source: Re = region, Zo = Zone, Si = Site. Abbreviations for posthoc tests: N = North, NE = Northeast, SE = Southeast, W = West; Eu = Eulittoral zone, Sub = Sublittoral Zone. Site abbreviations as in Figure 2.1.

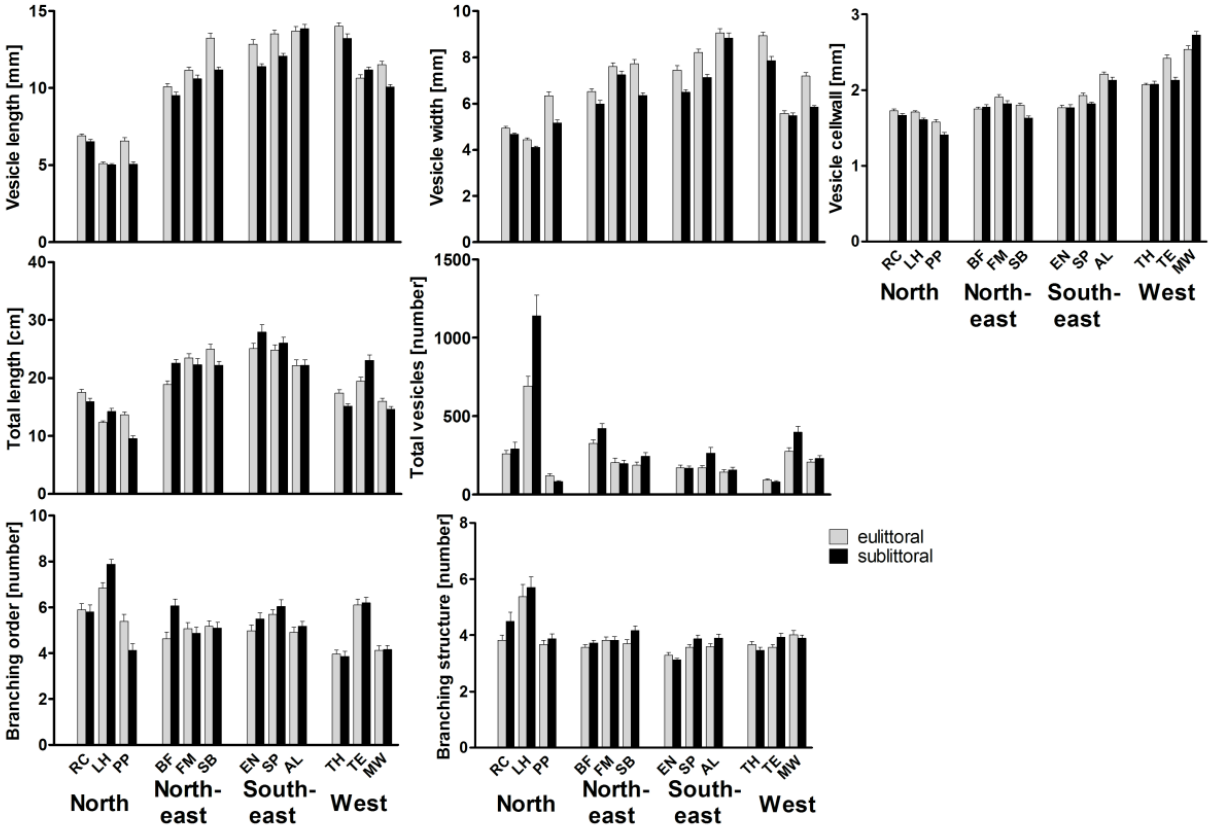

Figure 2.4: Mean (± SE) of seven morphological traits in *Hormosira* sampled in four regions (north, northeast, southeast, west), three sites within each region and two zones (sublittoral and eulittoral) at each site. Site abbreviations as in Figure 2.1. Each bar represents n=30 replicates.

Environmental variability

The three north region sites and one of the northeast region sites separated along PCO 1 reflecting the strongly semi-diurnal tidal regime of sites in the north region and the mixed, mostly semi-diurnal tidal regime at that northeastern site (Bay of Fires) (Figure 2.5). PCO 1 highlighted strong negative loadings for two tidal variables (tidal regime: r = -0.832; maximum tidal difference: r = -0.623) but strong positive loadings for sea surface temperature (SST: r = 0.720) and neap tide (r -0.728), whereas wave exposure (r = 0.827) and wave height (r = 0.886) had strong positive loadings along PCO 2 (Figure 2.5). Southport, Shelly Beach and Rocky Cape separated from the other sites along PCO 2 reflecting their low wave exposure and wave height while two west region sites (Trial Harbour and Marrawah) and to a lesser extent, one north region site (Petal Point) separated along the other end of that axis reflecting the higher wave exposure and wave height at those sites (Figure 2.5).

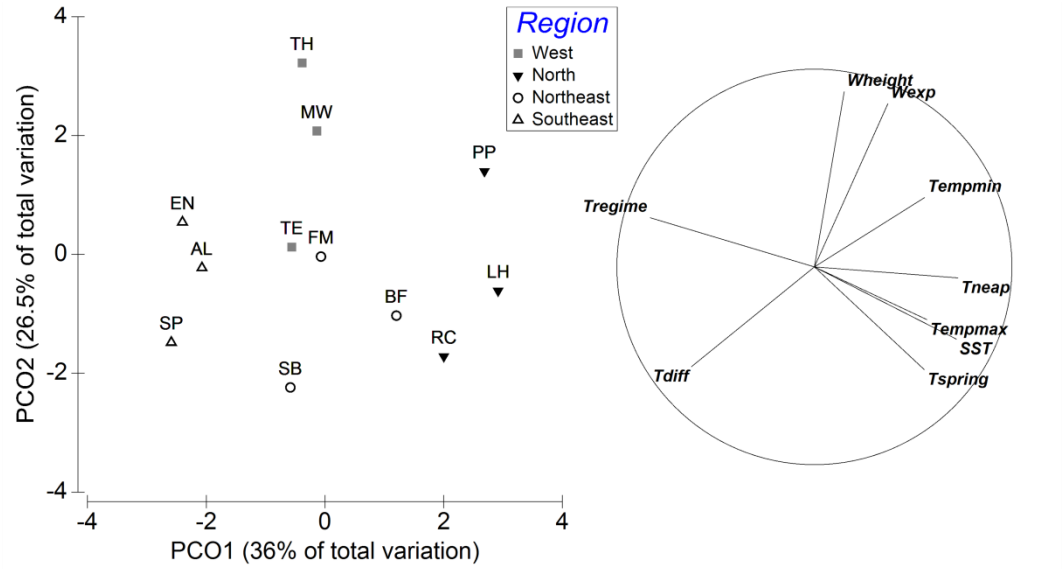

Figure 2.5: PCO ordination of environmental variation at three sites in each of the four regions. Site abbreviations as in Figure 2.1. Symbols correspond to collection region. PCO1 and PCO2 refer to the first and second principal coordinates axes and indicate the percentage of variation explained by each axis. Inset shows the vectors based on Pearson correlation for environmental variables (Tspring: maximum spring tide, Tneap: minimum neap tide, Tdiff: maximum tidal difference, Tregime: tidal regime, Wheight: max wave height, Wexp: wave exposure, SST: mean sea surface temperature, Tempmin: mean minimum air temperature 2011, Tempmax: mean maximum air temperature 2011).

Relationship between morphology and environment

All environmental variables showed a significant relationship with the multivariate morphological patterns (DISTLM, marginal tests, Table 2.4) although the amount that each variable contributed to explaining morphological patterns varied. The total variation explained by all environmental variables was 52.4 % (R^2 = 0.524). However, nearly half of this variation could be explained by fitting only one variable: tidal regime (R^2 = 0.231). A more parsimonious model with fewer predictor variables: spring tide, maximum tidal difference, tidal regime, maximum mean temperature and SST (variables: 1, 3, 4, 7, 9; Table 2.4; R^2 = 0.443) was used to fit

the dbRDA-model. This combination had the lowest AIC for five variables with little improvement if more variables were added (Table 2.4). The reduced DISTLM reinforced the relative importance of the tidal variables, especially the tidal regime, in explaining morphological variation in *Hormosira* and the low contribution of wave characteristics (wave height and wave exposure). The dbRDA using the most parsimonious model with five environmental variables showed a clear separation between sites from the north region (Petal Point, Low Head, Rocky Cape) and other sites along dbRDA 1 (Figure 2.6). DbRDA1 and dbRDA2 account for 89.5 % of the variability in the fitted model which corresponded to 39.7 % of the total variation in the morphology of *Hormosira*. Tidal regime was strongly negatively correlated with dbRDA 1 (r = -0.824), whereas spring tide (r = 0.751) and SST (r = 0.624) were positively correlated with axis 1. Thus, the separation of the north region sites from other sites along dbRDA1 highlights the importance of three environmental variables: semi-diurnal tidal regimes, high spring tides and high SST, in predicting the generally smaller morphology of *Hormosira* in the north, compared to other regions.

Table 2.4: DISTLM models. Marginal tests show the relative contribution of each variable tested individually. Variables: Tspring = maximum spring tide, Tneap = minimum neap tide, Tdiff = maximum tidal difference, Tregime = tidal regime, Wheight = max wave height, Wexp = wave exposure, Tempmax = mean maximum air temperature 2011, Tempmin = mean minimum air temperature 2011, SST = mean sea surface temperature.

Marginal tests				***Conditional tests***			
Variable	**SS (trace)**	**Pseudo-F**	**Prop.**	**AIC**	**R^2**	**No. Vars**	**Selected variables**
1. *Tspring*	964.55	**170.22 *****	0.191	1215.3	0.231	1	4
2. *Tneap*	1050.4	**189.37 *****	0.209	1122.3	0.326	2	2, 4
3. *Tdiff*	713.27	**118.56 *****	0.142	1059.9	0.383	3	2, 4, 6
4. *Tregime*	1160.8	**215.25 *****	0.231	1033	0.408	4	2, 4-6
5. *Wheight*	308.39	**46.867 *****	0.061	990.41	0.443	5	1, 3, 4, 7, 9
6. *Wexp*	285.75	**43.219 *****	0.057	963.23	0.465	6	1, 3-5, 7, 9
7. *Tempmax*	163.22	**24.066 *****	0.032	939.77	0.484	7	1, 3-5, 7-9
8. *Tempmin*	433.05	**67.594 *****	0.086	906.92	0.508	8	1-8
9. *SST*	657.45	**107.88 *****	0.131	885.83	0.524	9	1-9

Bold text refers to significant factor effects (asterisks represent the significance level: *** p < *0.001*, ** p < *0.01*, * p < *0.05*). Prop. describes the rise in the proportion of explained variation for each variable. Conditional tests show best results for each number of variables fit into the model based on AIC. The amount of explained variation is described via R^2. Selected variables (right column) refer to the order variables are listed in the marginal tests.

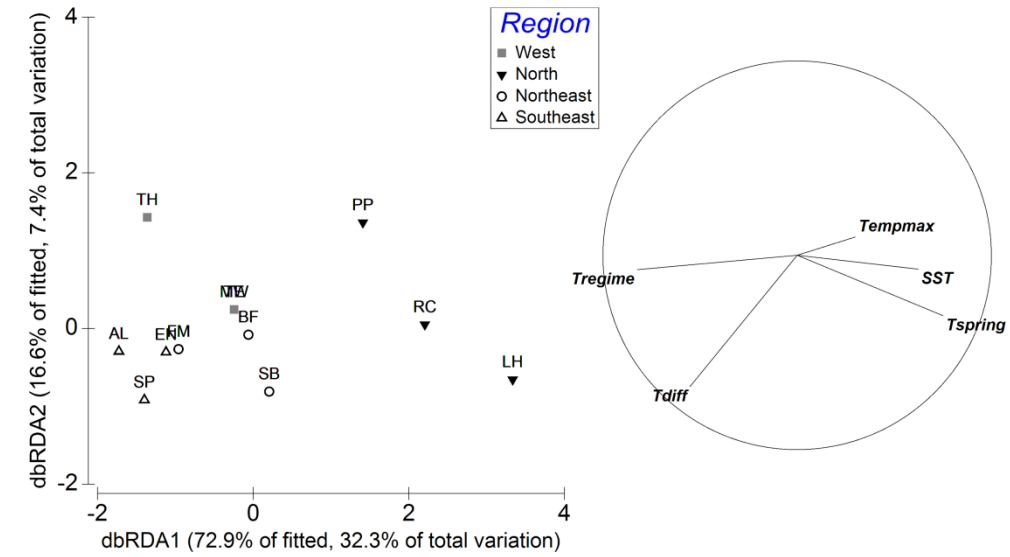

Figure 2.6: dbRDA ordination for the relationship between *Hormosira* morphology and environmental variation at each of three sites from each of the four regions. Inset shows the vectors based on Pearson correlation for environmental variables that contribute most towards explaining overall and fitted variation with the dbRDA axeis. Site abbreviations for sites as in table Figure 2.1. West coast sites Marrawah (MW) and Temma (TE) overlap in the plot.

Discussion

Adaptation to hydrodynamic environments has been widely described as a main cause for variation in the morphology of seaweeds (Hurd 2000, Wernberg et al. 2003). In this study, significant variation in *Hormosira banksii* morphology occurred at all spatial scales examined: between different geographical regions around Tasmania, between sites within those regions and between zones at most sites. In particular, the morphology of *Hormosira* from the north coastline of Tasmania was distinct, generally having smaller vesicles and shorter thalli compared to *Hormosira* from other regions, especially the northeast and southeast coast. This pattern occurred despite substantial morphological variation within and among the three north region sites. Although this region is subject to relatively low wave exposure, tidal variables were the main environmental factors separating north coast sites

from other sites and the dbRDA analysis showed they were the best predictors of morphological differences between regions.

Large-scale patterns

Compared to the rest of Tasmania, sites on the north coast are exposed to higher tidal amplitudes (spring and neap tides) and a semi-diurnal tidal regime. All other regions around Tasmania experience a mixed and in the case of the southeast coast a mixed, mainly diurnal regime where tides are characterised by a large inequality or variation in water levels in high and/or low water heights (De Boer et al. 1989). Tides cause changes in hydrostatic pressure, overall temperature (i.e. temperature in water versus temperature outside water), salinity, turbulence, current speed and direction (Thurman 2004) and thus there may be greater daily fluctuations in oceanographic conditions on the semi-diurnal north coast. However, the combination of the higher tidal amplitude and semi-diurnality means intertidal seaweeds at northern sites experience different emersion and submersion intervals compared to other regions which are likely to be important. In particular, *Hormosira* on the north coast will often be exposed to the air twice every 24 hours. Exposure to the air in intertidal seaweeds results in thermal and desiccation stress, an increased risk of tissue damage due to high UV light, lower photosynthetic rates, lower growth and lower abundance (Schonbeck and Norton 1980, Chapman 1995, Wright et al. 2004, Williams and Dethier 2005). Keough & Quinn (1998) found that *Hormosira* is susceptible to sunburn during periods of high UV-radiation and suggested that summer low tides are particularly stressful for *Hormosira* in southern Australia. During low tide in summer, *Hormosira* tissue appears brittle and damaged (Schoenwaelder 2002) which may affect reproduction and fitness (McKenzie and Bellgrove 2008). Other intertidal seaweeds also have reduced performance during summer low tides (Wright et al. 2004, Williams and Dethier 2005). The smaller size

and vesicle morphology of *Hormosira* on the north coast may make them less susceptible to tissue damage when exposed to the air for long time-periods during summer. This smaller morph creates a dense canopy at low tide which strongly reduces temperature and desiccation stress (R. D. Lewis, unpublished data). Small morphology or "dwarfism" is found in several other fucoids (Sideman and Mathieson 1985, Scott et al. 2001, Wright et al. 2004). Typically these small morphologies occur in the very high intertidal zones suggesting it may be a response to thermal stress and desiccation. However, a smaller morphology in *Fucus gardneri* also occurs in response to increasing wave exposure (Blanchette et al. 2002). We found no evidence for a smaller morphology in *Hormosira* at more wave exposed sites. However, the Bass Strait in the north coast bears the strongest tidal currents around Tasmania due to the high tidal range and a more constricted flow (Short 2006a). A smaller morphology results in less drag in seaweeds (Martone et al. 2012) and thus might be advantageous under the more frequent, high amplitude tidal conditions of the north coast.

Previous studies of *Hormosira* have shown a positive correlation between water loss and vesicle size, suggesting that larger vesicles have a higher desiccation tolerance (Bergquist 1959) and thus larger vesicles would be predicted at sites with a higher risk of desiccation (Ralph et al. 1998). Our data does not support this prediction. The semi-diurnal tidal regime at north coast sites would result in long emersion times and a high desiccation risk during summer yet these sites had the smallest vesicles during summer. Larger vesicles were found at west and east coast sites, where the lower tidal amplitude and mixed semi-diurnal tidal regimes would result in generally lower emersion times and a lower desiccation risk. Our west and east coast sites experience winter low tides either at night or early in the morning (Australian National Tides Tables 2013) and thus little low tide exposure during the

day. Low ambient light and short day length during winter may instead lead to light-limiting conditions and can affect photosynthesis (King and Schramm 1976) and carbon acquisition (Kübler and Raven 1994). Under those conditions, larger vesicles may be advantageous for the photosynthetic performance in *Hormosira*. *Laminaria saccharina* individuals with large thalli had higher photosynthetic yield than those with smaller thalli (Lüning and Dring 1985). Nonetheless, *Hormosira* thalli will have to cope with day-time emersion and desiccation during summer at those sites.

Although our analyses emphasised the importance of tidal parameters, regional-scale effects could possibly influence the observed patterns in this study. Other environmental variables may also contribute to large-scale morphological differences in *Hormosira*. Mean SST was higher at north coast sites than southeast and west coast sites. Surprisingly, there was no evidence that wave exposure and wave height contributed to morphological variation. *Hormosira* from sheltered estuaries and rock pools typically have larger vesicles than from open coasts (Ralph et al. 1998, Macinnis-Ng et al. 2005). We intentionally did not sample estuaries but a *Hormosira* morph with large vesicles does occur in an estuary near one of our sites (Low Head). Although the Baardseth index correlates well with wave height and maximum water velocity (Wernberg and Vanderklift 2010) it does not consider topography of the coastline nor main wind direction and thus, wave impact could have been underestimated in our study (Kalvas and Kautsky 1998).

In addition to environmental factors, the differences between regions may reflect genetic differences due to the isolation between them. The dominant boundary currents of the east and west coasts do not influence the north coast greatly and may result in limited gene flow and connectivity between those regions and the north coast. In fact, the Bassian Isthmus, a historical land bridge that connected northern

Tasmania to the southeastern tip of mainland Australia, has been emphasised as a phylogeographical barrier between eastern and western populations of several marine species (Kurth 1957, Dawson 2005, Waters 2008). When sea-levels rose at the end of the last glacial period this land bridge narrowed to a small isthmus to the eastern side (approximately 14000 BP) (Lambeck and Chappell 2001). Consequently, western populations of marine species were able to expand their range into the submerged northwest coast of Tasmania while eastern populations were still isolated by the narrow isthmus (Waters 2008). Our results suggest some evidence that *Hormosira* from west coast sites are separated from east coast sites (Figures 2 and 3) and are consistent with findings of morphological differences for *Durvillaea potatorum* between those coasts (Cheshire and Hallam 1989, Fraser et al. 2009).

Small-scale patterns

Our study showed significant small-scale variation in morphology among sites within regions (especially for the north region) and between zones within sites. These differences also involved dispersion effects between sites. Dispersion effects are often a good indicator of small-scale environmental variability (Anderson et al. 2008) and increased variability has been linked to stressful conditions (Warwick and Clarke 1993, Chapman et al. 1995). The large morphological variability within and among northern sites could be linked to variation in environmental conditions such as air tidal exposure, temperature and wave exposure (Table S1) possibly promoting local adaptation. Intertidal seaweeds are already being affected by increasing land temperatures (Bertness et al. 2006) and mean annual minimum air temperature varies substantially across northern sites (PCO 2, Figure 5). Without any major currents (except for tidal flows) fluid transport in the north relies only on wind drift (Baines et al. 1991). The coastal topography in the Bass Strait consists of

many headlands and spits (build-ups of sand stretches that are caused by waves) that act as barriers between sites (Woodroffe 2003) affecting the potential for dispersal (Kalvas and Kautsky 1998). As a consequence, small-scale connectivity between sites may be reduced. In contrast to the north coast, the presence of strong boundary currents on the east and west coasts are likely to promote high connectivity of *Hormosira* populations (Coleman et al. 2011b) as occurs for other seaweeds (Fraser et al. 2009). The similar gross morphology for *Hormosira* along Tasmania's east coast suggests that the EAC enhances dispersal and connectivity between sites. Although *Hormosira* gametes have limited dispersal, fertile thalli of *Hormosira* have the potential for long distance dispersal via drifting (McKenzie and Bellgrove 2008) and may be more important than gametes in maintaining connectivity.

Long day-time exposure to the air (especially in summer) strongly affects intertidal organisms (Schonbeck and Norton 1978). In intertidal seaweeds, photosynthesis is usually lower in the air (Williams and Dethier 2005) meaning individuals in high zones, with a greater exposure time, will acquire less carbon than individuals in lower zones. At most sites there were differences in *Hormosira* morphology between zones and although there was variation among sites, eulittoral individuals often had larger vesicles than sublittoral individuals. This suggests there may be some consistency in morphological response to different hydrodynamic and environmental regimes of the different zones (Wernberg et al. 2003). However, north coast morphology emphasizes the importance of tidal regimes rather than tidal height (and therefore different zones) as a possible explanation for morphological variation highlighting the likely role of a regional response to differing selective pressures.

In seaweeds, a number of studies have demonstrated the importance of environmental factors, in particular wave exposure, in shaping morphology (Kalvas & Kautsky 1993; Blanchette 1997; Kalvas & Kautsky 1998; Blanchette et al. 2002; Fowler-Walker et al. 2005; Wernberg & Vanderklift 2010). Although we did not measure all potential environmental factors, such as nutrient concentrations, our results highlight tidal conditions (especially different tidal regimes) as a potentially important contributor to morphological variation in *Hormosira* at multiple scales. Moreover, despite small-scale variation in morphology there were clear regional-scale differences. While the relative importance of environmental and genetic factors in determining *Hormosira* morphology remains unclear, this study highlights potential mechanisms underpinning phenotypic variation in complex environments in ecologically important seaweeds.

Chapter 3

Low levels of phenotypic plasticity highlights an important role for localised adaptation in a habitat-forming seaweed

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Introduction

Many organisms have ecotypes that differ in morphology across heterogeneous environments. Typically these ecotypes are thought to represent locally adapted phenotypes but they can also reflect plastic responses to local environmental conditions (Kleunen and Fischer 2001, Parmesan 2006, Lind et al. 2015). Spatially divergent selection can promote local adaptation (Zardi et al. 2013) resulting in local phenotypes having a higher fitness in their native habitat compared to foreign phenotypes from more distant habitats (Kawecki and Ebert 2004). The ability to express phenotypic plasticity is also of ecological importance if it increases individual fitness within the environment (Gerard et al. 1987, Via 1993) and is likely to be particularly beneficial for sessile species as they cannot escape environmental change after recruitment (Bradshaw 1972, Monro and Poore 2009).

Because both local adaptation and plasticity influence phenotypic differentiation within species (Pigliucci 1996), their effects can be determined via reciprocal transplant experiments between local and foreign sites (Kawecki and Ebert 2004, Reusch 2014) even though these experiments often bear complex logistics (Carpenter 1990). In addition, linking transplant experiments with measurements of key environmental variables among sites can provide evidence for the role of specific selective factors driving adaptive or plastic responses (Sanford and Kelly 2011).

Morphology is a composite measure (Wernberg and Thomsen 2005) and the optimal phenotype in an environment is a combination of traits (Fischer and Van Kleunen 2002). Individual morphological traits also vary in their plasticity (Wernberg and Thomsen 2005) and one trait can be regulated by plastic mechanisms while another trait may be under strict genetic control (Wernberg et

al. 2003). It is therefore crucial to explore the impact of environmental factors on both individual morphological traits and overall trait composition to understand the relationship between the multivariate phenotype and its environment. In addition, environmental conditions often vary at local scales (Coleman and Muhlin 2008, Zardi et al. 2013) and therefore studies need to address the possibility that population-level responses may be independent of regional responses (Dethier et al. 2005, Nicastro et al. 2010).

Morphological variation in terrestrial plants occurs in response to variation in a range of environmental factors including light (Donohue et al. 2000), aridity (Etterson 2004), altitude (Cordell et al. 1998), temperature (Williams and Black 1993) and landscape gradients (Walter et al. 2016). Similarly, in seaweeds, morphological variation often occurs across environmental gradients of wave exposure (Kalvas and Kautsky 1993, Thomsen et al. 2004, Bekkby et al. 2014), light (Lüning 1992, Wing et al. 2007), temperature (Mabin et al. 2013), salinity (Ruuskanen and Bäck 1999), flow (Koehl et al. 2008) and tidal regimes (Mueller et al. 2015). Distinct seaweed ecotypes are often attributed to variable wave exposure (Blanchette et al. 2002, Roberson and Coyer 2004) but there are few experimental tests for seaweeds to separate adaptive and plastic responses. The limited number of transplant experiments show some evidence for both responses but they appear species-specific; for example morphology appears fixed in *Eisenia arborea* (Roberson and Coyer 2004) and *Eregia menziesii* (Blanchette et al. 2002), but plastic in *Eklonia radiata* (Fowler-Walker et al. 2005) following transplantation to sites of different wave exposure.

Rocky intertidal habitats span steep environmental gradients and both marine and terrestrial stressors that act on intertidal communities can vary among sites. For

intertidal communities, exposure to terrestrial climatic conditions at low tide is a major stressor and both the duration and timing of emersion varies among sites (Helmuth et al. 2002, Williams and Dethier 2005). Emersion stress negatively affects photosynthesis (Williams and Dethier 2005), growth (Wright et al. 2004), development (Davison et al. 1993) and recruitment (Taylor and Schiel 2003) in intertidal seaweeds and leads to nutrient limitation and an increased risk of desiccation (Davison and Pearson 1996). Moreover, stress increases as emersion duration increases (Davison and Pearson 1996, Stengel and Dring 1997, Zardi et al. 2013) and stress can be intensified when low tide coincides with periods of extreme air temperatures (both warm and cold) (Helmuth et al. 2002).

The habitat-forming brown seaweed *Hormosira banksii* (Turner) Descaisne (Fucales, Phaeophyceae) forms dense canopies on intertidal shores of temperate Australasia. *Hormosira* shows large phenotypic variation across its distributional range and several ecotypes are recognised (Ralph et al. 1998, Macinnis-Ng et al. 2005, Mueller et al. 2015). On the north coast of Tasmania, an island situated south of mainland Australia, populations of *Hormosira* have a distinct bushy morphology with short fronds, small vesicles and high levels of branching. This bushy northern ecotype differs to populations from the adjacent east and west coasts which are characterised by a larger size with large vesicles and a low degree of branching and tidal regime is the best predictor for these morphological differences among coasts (Mueller et al. 2015) (Figure 3.1).

In this study, we determined whether environmental conditions promoted phenotypic change and influenced performance in different ecotypes of *Hormosira banksii* between the north and east coasts of Tasmania. Initially, we quantified how variation in tidal regimes between coasts influenced temperature fluctuations and

emersion time at sites to gain insight into selective factors driving the responses. We then used a reciprocal transplant experiment and transplanted north and east coast morphs to the alternative coast and to a nearby site within each coast. In particular, we tested whether i) individuals transplanted to a different region or site retained the morphology of individuals at their origin (e.g. morphology is fixed, indicated by an effect of origin) or ii) individuals transplanted to a different region or site developed the morphology of native individuals at their destination (e.g. morphology is plastic, indicated by an effect of destination). We further determined whether growth rates differed when individuals were transplanted away from their site of origin to test for an advantage of native phenotypes in their local environment.

Material and Methods

Study species and system

Hormosira banksii can grow up to 300 mm in length (Edgar 1997) from a single holdfast via apical growth (Clayton 1984). Fronds (one or multiple per thalli) grow slowly (Clarke and Womersley 1981) and consist of a chain of water-filled vesicles (Osborn 1948). Vesicles are linked by connectives (Osborn 1948, Bergquist 1959, Clarke and Womersley 1981, Macinnis-Ng et al. 2005) creating variable forms of branching. Conceptacles, located on vesicles contain gametes which are released on incoming tides, resulting in year-round recruitment (Bergquist 1959). Zygotes are direct-developing and usually settle in close proximity to their parental generation resulting in limited dispersal (McKenzie and Bellgrove 2006).

The study was conducted at two sites within each of two regions in Tasmania, Australia: the north and the east coast. Although there is some variation among sites

within regions, *Hormosira* from the northern and eastern regions possess very different morphologies; smaller and highly branched thalli on the north coast compared to elongated, less branched thalli on the east coast (Figure 3.1; Mueller et al. 2015). While both regions have some wave exposure, wave dynamics are stronger on the east coast (Short 2006a). Tasmania's north coast faces Bass Strait, a shallow water basin without any major currents that is protected from swell by the Australian mainland and mostly subject to wind-driven waves (Short 2006a). In contrast, the Tasmanian east coast is exposed to swells from the northeast, east and southeast directions. The north coast is also exposed to a semi-diurnal tidal regime and experiences higher tidal amplitudes compared to a mixed diurnal tidal regime at the east coast (McInnes et al. 2011). These differences in tidal regime likely result in differences in emersion (both the amount of emersion and its timing) between the coasts. The two north coast sites at Beechford (N1; 41°01'22"S, 146°56'39"E) and Bell Buoy Beach (N2; 41°02'23"S, 146°49'56"E, Figure 3.1) consisted of intertidal rock-flats characterised by exposed cobble-basalt headlands (Short 2006a). The two east coast sites were Sloop Reef (E1; 41°12'17"S, 148°16'53"E) and Four Mile Creek (E2; 41°33'26"S, 148°17'35"E); both are wave-dominated systems along sloping granite boulder headlands (Short 2006a). At all four sites *Hormosira* is the dominant seaweed in the intertidal zone.

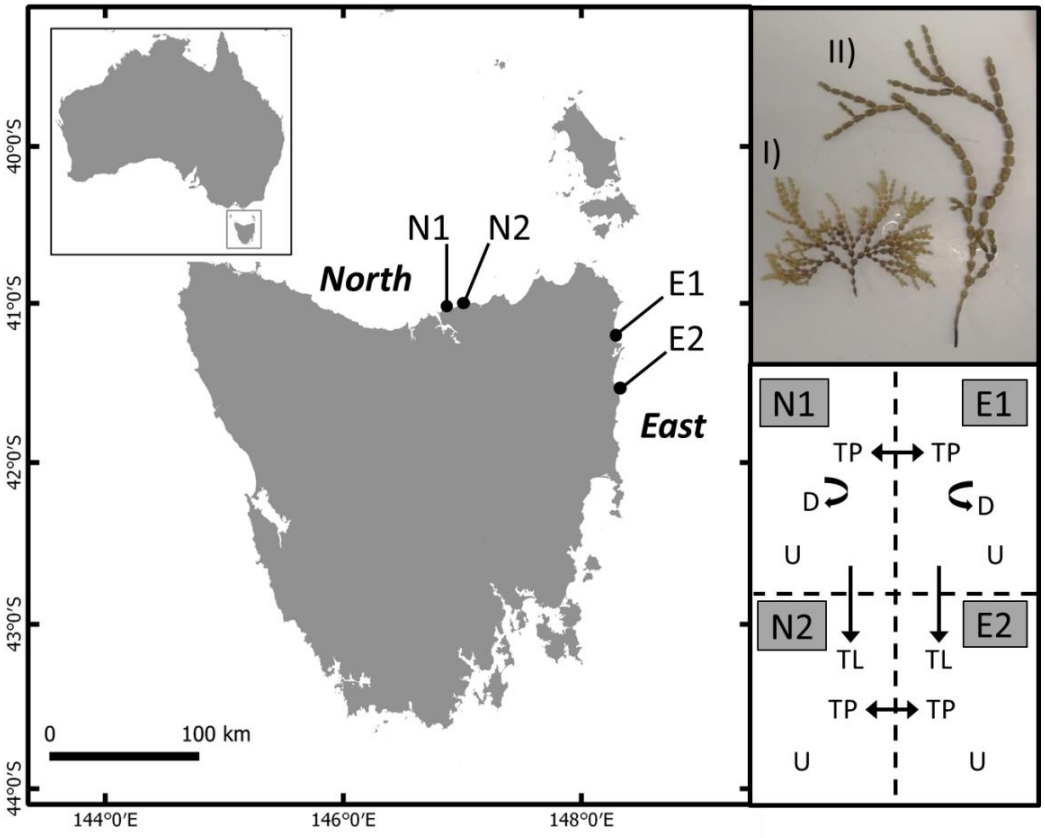

Figure 3.1: Map of Tasmania showing the locations of the two sites on the north and east coasts. N1 (Beechford) and E1 (Sloop Reef) were the sites used in experiment 1 while N2 (Bell Buoy Beach) and E2 (Four Mile Creek) were the sites used in experiment 2. Picture (top right) shows typical representative forms for each region: I) north coast morph and II) east coast morph. Schematic inset (bottom right) displays the treatments established at each location (undisturbed, U; disturbed, D; translocated, TL; transplanted, TP).

Temperature and emersion regimes

Temperature at each site was logged every 10 minutes throughout the experiment using a HOBO ® TidbiT v2 data logger (Onset computer corporation, Bourne, MA, USA). At each site two temperature loggers were attached to the rocky substrate with epoxy beneath the *Hormosira* canopy adjacent to the transplants. Temperature was averaged across the two loggers for each site. We characterised temperature regimes at each site based on temperature fluctuations to describe patterns of

thermal variability. Temperature fluctuations were defined as maximum deviation in temperature from the 12 h mean temperature over a period for which the actual temperature deviates more than 1 °C from the 12 h mean temperature. Any temperature fluctuations less than 1 °C were considered as zero deviation. We numerically estimated emersion time at each site for the *Hormosira* transplants (i.e. hours per day that the thalli were exposed to the air) and summed these for each month to give an indication of the total exposure time per month. This was done by integrating time intervals (of 10 min each) for which the forecasted chart datum (tidal height) correlated to exposed individuals based on their relative height at each site.

Reciprocal transplant experiment

Transplantation between north and east coast regions was done in two experiments. Both experiments tested the same main question: whether morphology in *Hormosira* was plastic or fixed. Together, the experiments allowed replication of sites without requiring the full reciprocal experimental design (and procedural controls) across all sites. Experiment 1 involved reciprocal transplantation between N1 and E1, while experiment 2 involved reciprocal transplantation between N2 and E2. All transplants were unbranched juvenile thalli at a similar development stage (4-7 vesicles, 4.16 ± 0.13, mean ± SE; approx. 20 mm in length, 19.5 ± 0.8, mean ± SE). In April 2014, juveniles were haphazardly collected from beneath the adult *Hormosira* canopy along a 10 metre transect placed at the centre of distribution for *Hormosira* at each site. We chose the centre of distribution for each population as a measure of standardisation due to differences in topography and tidal range for each region. In experiment 1, the sites N1 and E1 had the following treatments 1) transplanted individuals (TP; n = 30): that were reciprocally transplanted between regions n N1 and E1; 2) translocated individuals (TL; n = 30): removed and

translocated to a different site within the same region (N1 translocated to N2, E1 translocated to E2); 3) disturbed individuals (D; n = 30): removed and reattached at the same site; 4) undisturbed individuals (U; n = 20): marked *in situ* at each site but otherwise left untouched. Undisturbed individuals were chosen haphazardly along the transect. A higher number of individuals were used for TP, TL, and D treatments to buffer against expected higher initial loss after transplantation.

In experiment 2, sites N2 and E2 had the following treatments 1) transplanted (TP; n = 30): individuals reciprocally between N2 and E2 and 2) undisturbed (U, n = 20) (Figure 1). This asymmetrical design allowed us to test the hypothesis with the required procedural controls (see Chapman 1986) but did not require all possible pairwise comparisons which was important given the short periods of low tide at some of these sites which restricted time for set-up and measurements. Comparing the undisturbed vs. disturbed vs. translocated treatments allowed a test for handling effects (removal from substrate, transportation and short-term storage at the laboratory, and reattachment to the new position) in each region regardless of the environment (Underwood et al. 2004). Two sets of discrete transplants between regions allowed us to test the main hypothesis of interest (the influence of environmental and genetic effects on morphology) by comparison between undisturbed vs. transplanted treatments. Two different types of analysis were performed for the main hypothesis, one from the point of view of destination and one from the point of view of origin of the alga.

Juvenile *Hormosira* were carefully scraped off the substrate ensuring that no parts of holdfast or frond were damaged. It was not possible to remove, measure, and return juveniles to corresponding treatments within one day. Consequently, individuals were transported to the lab and kept in water with aeration at 17˚C on a 12:12 light

regime until transplanted (a maximum of 3 days depending on travel time between sites). *Hormosira* were reattached to the substrate using pieces of coiled polypropylene rope (approximately 8-9 cm in length, diameter 6mm). Rope strands were uncoiled in the middle section, and the juvenile holdfast inserted through the coils so that the holdfast protruded on one side and vesicles on the other. Thalli were secured to the rope by re-twisting the rope closed. Both ends of the rope were then anchored to the rock substrate with underwater epoxy (A-788 Splash Zone, Z-SPAR, US) beneath the *Hormosira* canopy with individuals transplanted along the same 10m transect from which juveniles were collected at the recipient site. Attachment of *Hormosira* was only possible during a certain time frame when the tide was low enough to give the epoxy sufficient time to cure (approximately one hour for transplants to withstand the incoming tide forces). All treatments that were removed and reattached (TP, TL and D) were treated in the same way.

Morphology

Prior to out-planting, all juveniles were photographed against a scale and then again *in situ* approximately every month for the first six months and final photographs were taken after 12 months. Seven morphological traits reflecting overall size and shape of fronds were measure from images using the image processing software ImageJ (National Institutes of Health, Bethesda, MD, USA). These traits were: 1) the total frond length (total length), 2) the total number of branch points per frond (total number of branching), 3) the highest hierarchical order of branching on frond (branching order), 4) the maximum number of connectives coming from a vesicle (branching structure), 5) the total number of vesicles per individual, 6) mean vesicle length and 7) mean vesicle width. Mean vesicle length and width were measured from three randomly chosen non-branched vesicles (excluding basal and apical

vesicles). Sample size differed among treatments as several juveniles were lost over the duration of the experiment.

Relative growth rates

To determine performance as a function of the environment, we measured relative growth rates (RGR) in terms of total frond length and total number of vesicles for the duration of the experiment (Monro and Poore 2009). The RGR was calculated as [ln (final measurement) – ln (initial measurement)]/number of days (Evans 1972). We used these parameters to test for local adaptation as indicated by higher growth of local compared to foreign individuals at each recipient site (Local vs. Foreign) and higher growth of individuals at their local environment compared to the different environment they were transplanted to (Home vs. Away) (e.g. Kawecki and Ebert 2004). For the sites E1 and N1 with U, TL and TP treatments, we also determined whether populations showed reduced RGR when transplanted outside their local site regardless of whether they were transplanted within their native (e.g. similar) region or to a different region.

Statistical analyses

Variation in the morphology of *Hormosira* between different treatments was compared using permutational multivariate analysis of variance (PERMANOVA; Anderson 2001). Multivariate analyses were based on a Euclidean-distance matrix with square root transformed and normalized data and used 9,999 permutations of residuals under the reduced model. Because our design was unbalanced we choose type III sums of squares to ensure full independence of model terms (Anderson et al. 2008). Significant factor effects were examined with post-hoc pairwise comparisons using 9,999 permutations under the full model with no corrections for inflated type I error rates because P-values gained under permutation test each null hypothesis

individually (Anderson et al. 2008). In cases where low numbers of unique permutations (i.e., < 100) were obtained, Monte-Carlo P-values were applied as they provide a more precise test statistic (Anderson et al. 2008). We assessed effect sizes for each model component as the percentage of the square rooted estimated components of variation (Anderson et al. 2008). Because of an unbalanced design results from an unequal n (due to the loss of juveniles) we re-ran the analysis with differently swapped orders of the factor terms and the results did not change. To assess whether some groups were morphologically more variable than others, we tested for differences in multivariate dispersion using the PERMDISP routine (Anderson et al. 2008). This routine further provides a tool to test for homogeneity among treatments. Multivariate differences in morphology between treatment levels for each region were shown with nonmetric multidimensional scaling (nMDS) based on calculated centroids for treatments at each site. Individual morphological traits were analysed with ANOVA with a single dependent variable based on Euclidean distances, which results in similar F ratios to traditional ANOVA, but avoid violation of the assumptions of ANOVA (Anderson et al. 2008). Data were 4^{th} root transformed to minimise effects of dominant values before Euclidean distances were calculated. All morphological analyses were performed using PRIMER 6 + PERMANOVA (Clarke and Gorley 2006, Anderson et al. 2008).

Test for handling effects: To determine whether handling affected morphology we ran a 1-factor PERMANOVA comparing the treatment (fixed) levels (U, D, TL) for each region (north, east). No handling effect would be evident if there was no difference between the U, D and TL treatments.

Test for origin effect: To determine the influence of origin in determining subsequent morphology we ran PERMANOVAs comparing U and TP treatments from

the same origin for overall phenotype and each individual trait. If morphology was determined by the environment (i.e. traits are plastic) then individuals transplanted to the other region would differ from individuals left undisturbed at their site of origin and there would be a significant treatment x region interaction (Figure 3.2). Alternatively, if morphology was genetically controlled (i.e. traits are fixed) then individuals transplanted to the other region would retain similar morphology to undisturbed individuals at their site of origin and there would be a significant main effect for region (Figure 3.2). These PERMANOVAs had three factors: treatment (fixed) with two levels (U, TP), experiment (fixed) with two levels (1, 2) and region of origin (random) with two levels (N, E).

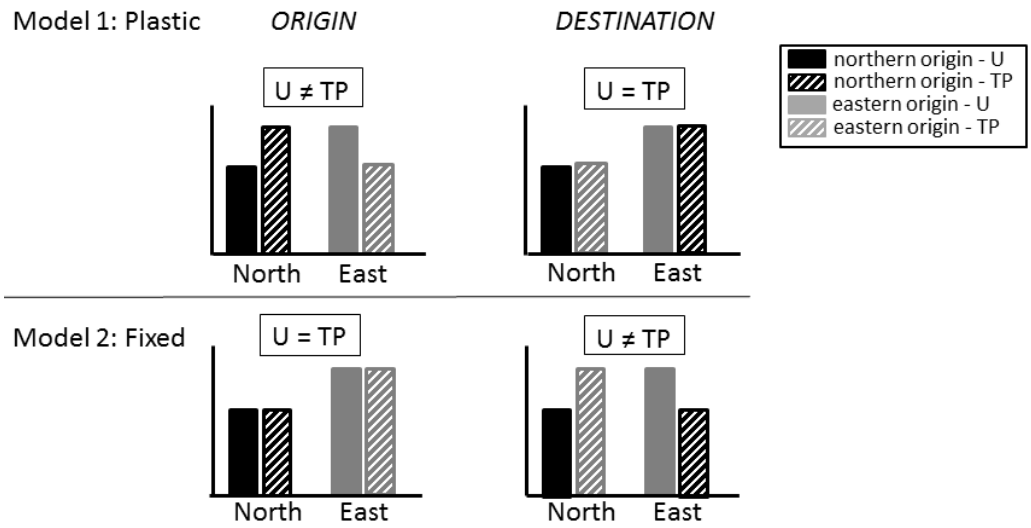

Figure 3.2: Predictions from the two models of the reciprocal transplant experiment for the test of origin and destination. Black bars indicate northern origin and grey bars refer to eastern origin. Unhatched bars represent undisturbed (U) groups while hatched bars represent transplanted (TP) groups.

Test for destination effect: To examine the effect of destination in determining the subsequent morphology we compared the overall phenotype and individual traits between U and TP treatments at each of the destinations. The test of destination

predicted that if morphology was influenced by the environment then individuals transplanted to the other region would have similar morphology to individuals left undisturbed at the destination site and there would be a significant main effect of region (Figure 3.2). Alternatively, if morphology was fixed then individuals transplanted to the other region will differ from undisturbed individuals at the destination site and there would be a significant treatment x region interaction (Figure 3.2). These PERMANOVAs had three factors: treatment (fixed) with two levels (U, TP), experiment (fixed) with two levels (1, 2) and region of destination (random) with two levels (N, E).

For tests of both origin and destination, the direction of the response may differ between populations within each region. This effect would be shown by significant region x treatment x experiment interactions for each test.

Relative growth rates: To compare RGR between local and foreign *Hormosira* at each site we used a PERMANOVA with the factors: Treatment (fixed) with two levels (Local, Foreign) and Site (random) with four levels (N1, N2, E1, E2). Second, to compare RGR in *Hormosira* at their local site vs. the site in the other region, we used a PERMANOVA with the factors: Treatment (fixed) with two levels (Home, Away) and Site (random) with four levels (N1, N2, E1, E2). Third, to compare RGR in *Hormosira* at their local site, a non-local but native site in the same region and a site in the other region, we used a PERMANOVA with the factors: Treatment (fixed) with three levels (Home, Native, Away) and Site (random) with two levels (N1, E1) as translocated treatments were only included in experiment 1.

Results

Temperature and emersion

The average temperature profiles at sites on the north and east coasts were similar with peaks in summer of approximately 19-20° C and winter of 12-13°C although extreme temperatures (reflecting periods of exposure to the air) were more evident on the north coast (Figure 3.3). When plotted as deviations from the mean temperature, both north coast sites showed greater temperature fluctuations than east coast sites (Figure 3.4). On the north coast there were generally 2-4 times as many cycles when the temperatures deviated by ≥ 5 °C above (N1, 44 cycles; N2, 40 cycles) and ≥ 5 ° C below (N1, 41 cycles; N2, 18 cycles) the average compared to the east coast (≥ 5° C above: E1, 20 cycles; E2, 12 cycles; ≥ 5 ° C below: E1, 6 cycles; E2, 14 cycles; Figure 3.4). The more frequent warmer and colder temperatures encountered on the north coast resulted in thermal changes in excess of 10° C within 24 hours on numerous occasions (Figure 3.4 A and B). In comparison, sites on the east coast showed remarkably little temperature deviation during winter and had prolonged periods without any temperature fluctuations (Figure 3.4 C and D). Small scale differences in temperature fluctuations between sites within both regions were also evident (Figure 3.4).

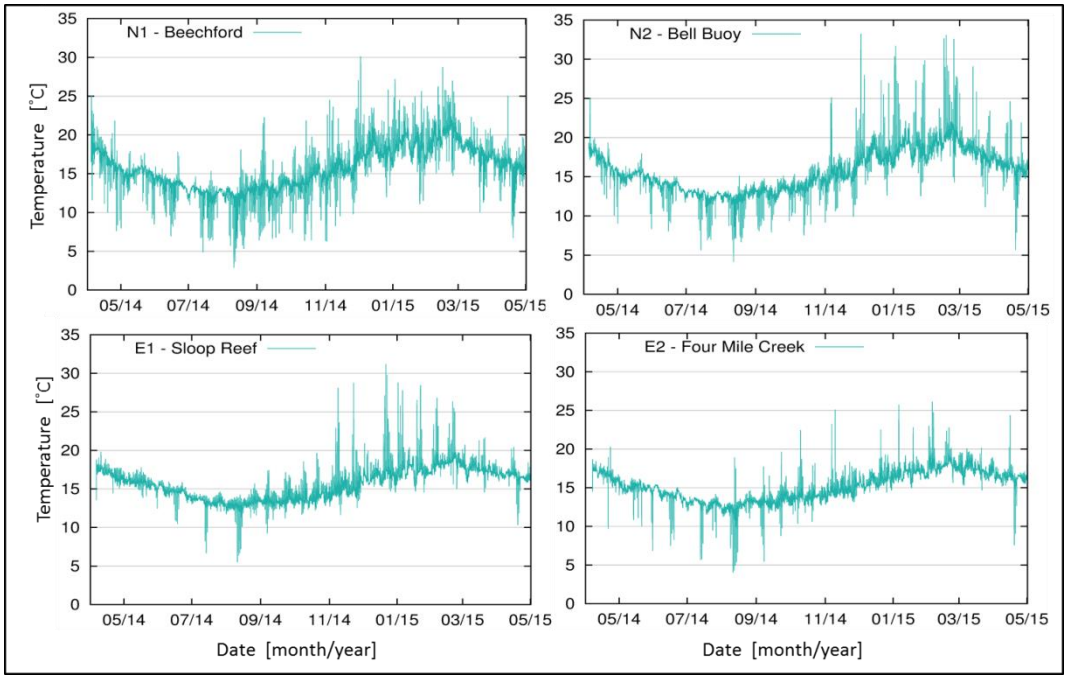

Figure 3.3: Temperature readings from beneath the canopy for the duration of the transplant experiment showing N1 (top left), N2, (top right), E1 (bottom left), and E2 (bottom right). Each plot represents data for one site averaged from two data loggers that were attached under the canopy.

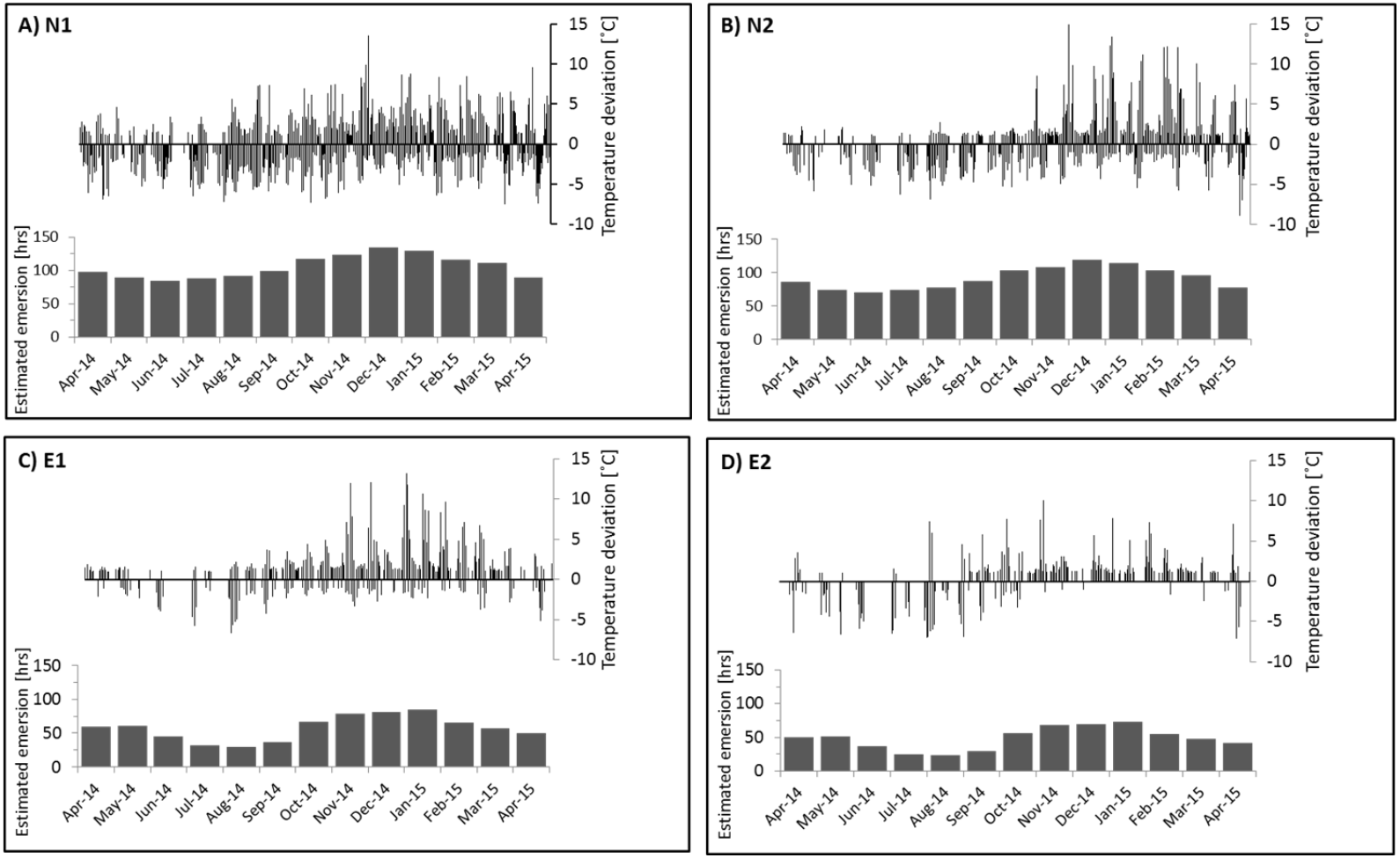

Figure 3.4: Temperature fluctuations and estimated emersion time for the duration of the transplant experiment at the four sites A) N1, B) N2, C) E1 and D) E2. Fluctuations were calculated as temperature deviation of more than ± 1 ˚C from the mean for each half tidal cycle (12 hrs). Estimated emersion times were plotted per month and show the cumulative time (hrs) during which experimental plots were exposed to air.

Although all sites followed a similar seasonal pattern in the amount of time exposed to the air per month with the least emersion from June – September and the highest from December – March, the north coast sites had approximately double the overall emersion reflecting the semi-diurnal tidal cycle on the north coast (Figure 3.4). From December – March, north coast sites had between 75 – 130 hours of emersion per month compared to east coast sites which had between 50 – 75 hours per month (Figure 3.4). Similar differences occurred between June – September with 70 – 100 hours of exposure per month on the north coast compared to 25 – 45 hours of exposure per month on the east coast. The greater emersion on the north coast between December – March coincided with the warmer months of the year when peak temperatures occurred during low tide. As with temperature fluctuations, emersion times also varied between sites within regions with slightly greater exposure times at N1 and E1 compared to the other respective site at each region (Figure 3.4).

Overall morphology

Handling had no significant effect on overall morphology and no differences were found between undisturbed, disturbed and translocated individuals within each region (north: $F_{2,16} = 1.15$, $P = 0.33$; east: $F_{2,21} = 0.82$, $P = 0.57$). The test for the effect of origin indicated that in general, individuals transplanted away from their native site did not change in overall morphology. The morphology of *Hormosira* from only one site (E1) differed significantly between undisturbed and transplanted individuals (significant treatment x region x experiment interaction and pairwise comparisons, Table 3.1A, Figure 3.5). The pairwise comparisons for N1, N2, and E2 were all non-significant (Table 3.1A, Figure 3.5). Morphology differed between populations of northern and eastern origin and accounted for 28.1% of total

variation (Table 3.1A, Figure 3.5). PERMDISP did not detect any differences in dispersions among samples ($F_{7, 53}$ = 0.75, P = 0.80).

Table 3.1: PERMANOVA testing for differences in the overall morphology of *Hormosira* between treatments, regions and experiments based on the point of view of A) origin of algae and B) destination of algae. For both analyses treatment was fixed with two levels (undisturbed, U; transplanted, TP), region was random with two levels (north, east) and experiment was fixed with two levels (1, 2). Effect size is given in percent and shows the contribution of each factor to the components of variation. Site abbreviations as in Figure 3.1

Source of variation	df	MS	Pseudo-F		effect size	Pairwise test	
A) Origin							
Treatment (Tr)	1	17.12	1.09		2.74		
Region (Re)	1	150.9	**45.5**	***	28.13	north ≠ east	
Experiment (Ex)	1	25.75	2.43		9.01		
Tr x Re	1	15.73	**4.74**	**	11.53	north: U = TP	
						east: U ≠ TP***	
Tr x Ex	1	1.32	0.07		0.0		
Re x Ex	1	10.60	**3.19**	*	8.84	N1 ≠ N2***	
						E1 = E2	
Tr x Re x Ex	1	18.65	**5.63**	**	18.15	level: Tr	level: Ex
						N1: U = TP	U-N1 ≠ U-N2*
						N2: U = TP	U-E1 ≠ U-E2*
						E1: U ≠ TP***	TP-N1 ≠ TP-N2*
						E2: U = TP	TP-E1 = TP-E2
Residual	53	3.32			21.60		
B) Destination							
Treatment (Tr)	1	17.12	0.11		0.0		
Region (Re)	1	15.73	**4.74**	**	8.07	north ≠ east	
Experiment (Ex)	1	25.75	1.38		6.09		
Tr x Re	1	150.94	**45.5**	***	39.38	north: U ≠ TP***	
						east: U ≠ TP***	
Tr x Ex	1	1.32	0.12		0.0		
Re x Ex	1	18.69	**5.63**	**	12.71	N1 = N2	
						E1 ≠ E2***	
Tr x Re x Ex	1	10.60	**3.19**	*	12.38	level: Tr	level: Ex
						N1: U ≠ TP***	U-N1 ≠ U-N2*
						N2: U ≠ TP**	U-E1 ≠ U-E2**
						E1: U ≠ TP***	TP-N1 = TP-N2
						E2: U ≠ TP**	TP-E1 ≠ TP-E2*
Residual	53	3.32			21.39		

Bold indicates statistical significance of factor effects (asterisks refer to significance level: *** P < *0.001*, ** P < *0.01*, * P < *0.05*). Sample size was n = 4-12.

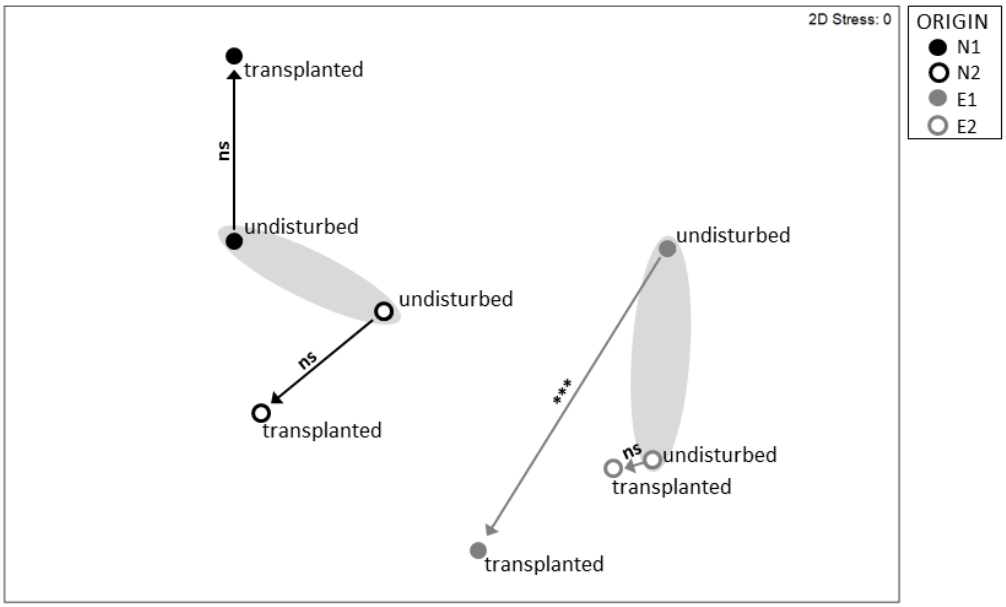

Figure 3.5: nMDS of *Hormosira* morphology based on centroids calculated for each treatment at each site. Data is shown from the point of view of origin. The grey shaded ellipses enclose undisturbed groups from the same region; arrows link undisturbed and transplanted groups originating from the same location (asterisks refer to significance level: *** P < *0.001*, ** P < *0.01*, * P < *0.05,* ns = non-significant). Site and treatment abbreviations as in Figure 3.1.

The test for the effect of destination showed that individuals transplanted to a different region did not develop a similar overall morphology to the undisturbed population at the destination. The overall morphology of transplanted individuals differed from the undisturbed populations at all destination sites (significant treatment x region interaction) and accounted for 39.4% of total variation (Table 3.1B, Figure 3.5). The analysis also detected differences in the response of populations to treatments between regions and experiments (significant treatment x region x experiment interaction) and post-hoc comparisons showed that individuals transplanted to the east coast differed in overall morphology between experiments (TP to E1 ≠ TP to E2) while individuals transplanted to the north coast did not differ in their overall morphology between experiments (TP to N1 = TP to N2). Also

overall morphology of undisturbed individuals varied between experiments in both regions (U-N1 ≠ U-N2; U-E1 ≠ U-E2) highlighting small-scale variation in morphology.

Individual morphological traits

The test for the effect of origin for individual traits showed different responses with some traits changing in transplanted individuals compared to undisturbed individuals at their site of origin, in particular *Hormosira* transplanted from E1 to N1. Total frond length declined in individuals transplanted from E1 to N1 and N2 to E2 compared to their undisturbed individuals ($F_{1,53}$ = 17.97, P < 0.001; post-hoc: both P < 0.05) (Figure 3.6). The total number of vesicles declined in individuals transplanted from E1 to N1 ($F_{1,53}$ = 15.16, P = 0.011; post-hoc: P < 0.001) while the mean length of vesicles declined in individuals transplanted from both eastern sites ($F_{1,53}$ = 9.76, P = 0.002; post-hoc: P < 0.001) (Figure 3.7). A similar trend was observed for mean vesicle width although this was not significant ($F_{1,53}$ = 2.06, P = 0.16) (Figure 3.7). Few changes occurred in branching related traits after transplantation and individuals grew similarly to those at their respective site of origin (Figure 3.8). Only individuals transplanted from N1 increased branching number when moved to the east coast ($F_{1,53}$ = 4.3, P = 0.04; post-hoc: P < 0.01) (Figure 3.8). Generally, branching was higher in northern origin individuals and this characteristic was retained when transplanted to the east coast (Figure 3.8).

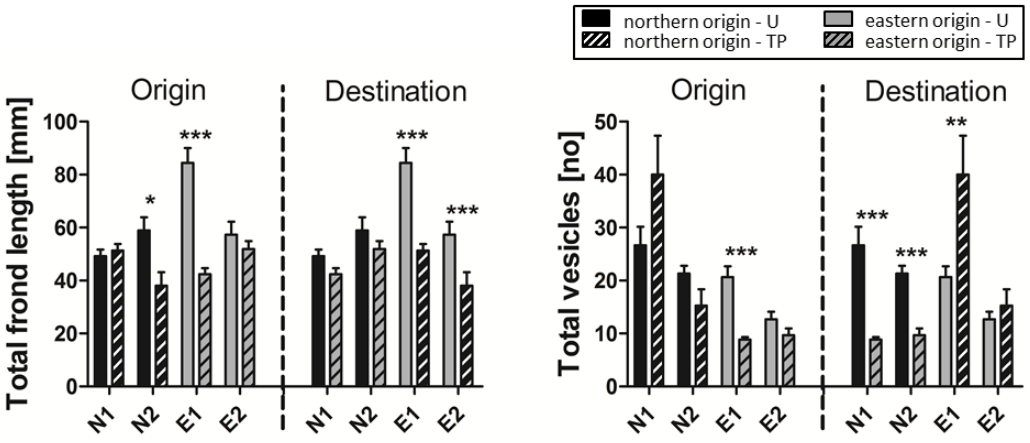

Figure 3.6: Mean (± SE) of frond related morphological traits in *Hormosira* measured in the transplant experiments for the different treatments for origin and destination effects (separated by dashed line). Black bars indicate northern origin and grey bars refer to eastern origin. Unhatched bars always represent undisturbed groups while hatched bars represent transplanted groups. Asterisks refer to significance level: ***P<*0.001*, **P<*0.01*, *P<*0.05*). Abbreviations as in Figure 3.1.

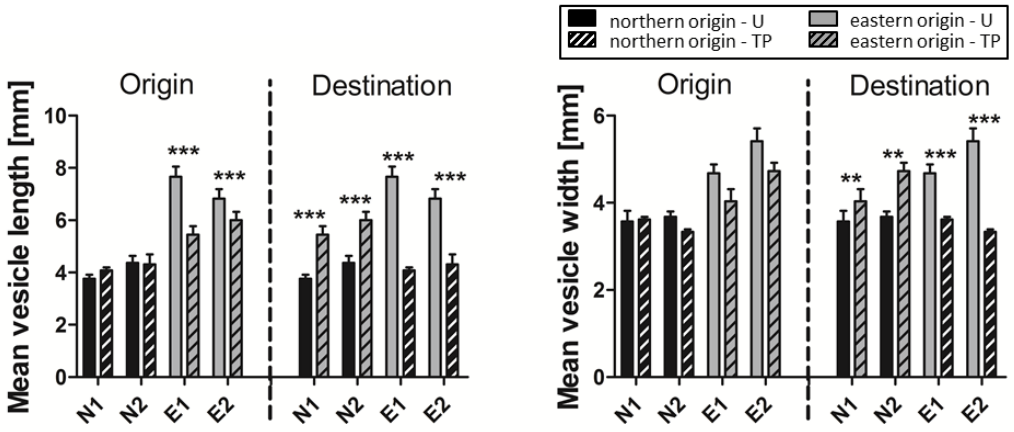

Figure 3.7: Mean (± SE) of vesicle related morphological traits in *Hormosira* measured in the transplant experiments for the different treatments for origin and destination effects (separated by dashed line). Black bars indicate northern origin and grey bars refer to eastern origin. Unhatched bars always represent undisturbed groups while hatched bars represent transplanted groups. Asterisks refer to significance level: ***P<*0.001*, **P<*0.01*, *P<*0.05*). Abbreviations as in Figure 3.1.

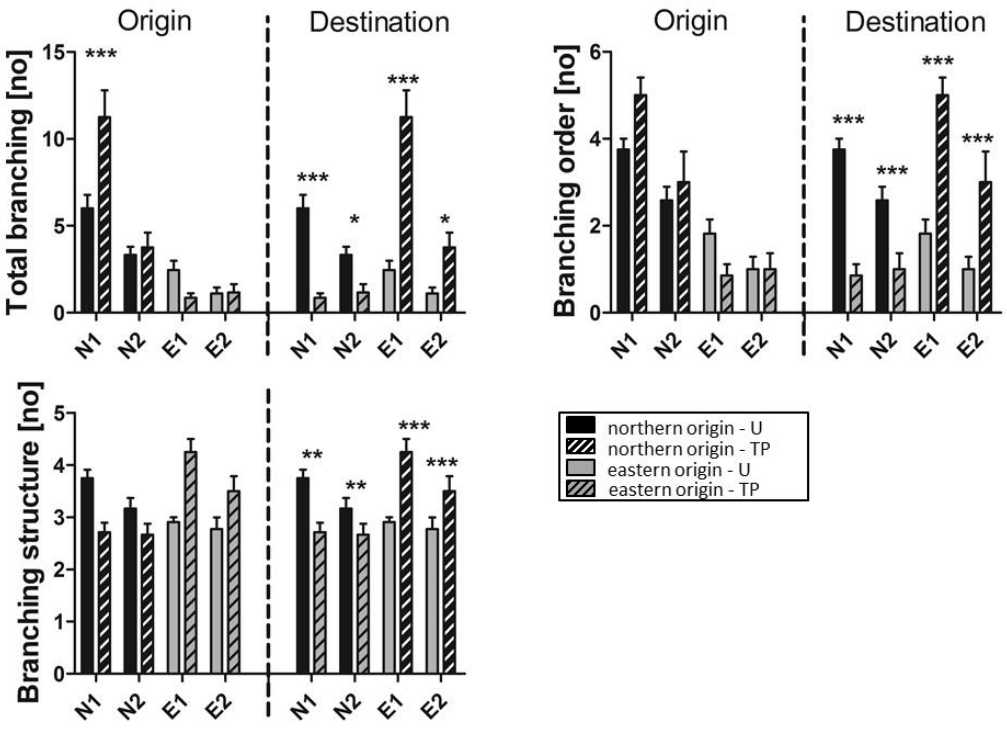

Figure 3.8: Mean (± SE) of branching related morphological traits in *Hormosira* measured in the transplant experiments for the different treatments for origin and destination effects (separated by dashed line). Black bars indicate northern origin and grey bars refer to eastern origin. Unhatched bars always represent undisturbed groups while hatched bars represent transplanted groups. Asterisks refer to significance level: ***P<*0.001*, **P<*0.01*, *P<*0.05*). Abbreviations as in Figure 3.1.

Consistent with the results for overall morphology, most traits differed between transplanted and undisturbed individuals at the destination site. Branching related traits were usually greater in undisturbed individuals on the north coast compared to eastern individuals transplanted to the north coast (total number of branching: $F_{1,53} = 65.93$, $P < 0.001$; branching order: $F_{1,53} = 44.57$, $P < 0.001$; branching structure: $F_{1,53} = 32.91$, $P < 0.001$; all post-hoc: $P < 0.01$) (Figure 3.8). The opposite was found at east coast destinations where northern individuals transplanted to the east coast had greater branching compared to native eastern populations (all post-hoc: P <

0.001). Frond length was similar between transplanted individuals moved from east to north vs. undisturbed northern individuals but at east coast destinations undisturbed individuals had larger fronds than the individuals transplanted from north to east ($F_{1,53}$ = 7.03, P = 0.01; post-hoc north: P = 0.12; post-hoc north: P < 0.001) (Figure 3.6). The total number of vesicles was higher in undisturbed individuals on the north coast compared to individuals transplanted from the east to north coast while transplanted juveniles from N1 to E1 had more vesicles than undisturbed individuals at E1 ($F_{1,53}$ = 6.94, P = 0.013; post-hoc: P < 0.01) (Figure 3.6). Vesicle length differed at the north coast and vesicles were larger in individuals moved from east to north but the opposite was true at the east coast where vesicles in individuals transplanted from north to east were significantly smaller compared to undisturbed eastern individuals ($F_{1,53}$ = 91.51, P < 0.001; post-hoc: P < 0.001) (Figure 3.7). Mean vesicle width at the north coast only varied between individuals transplanted from E2 to N2 and the undisturbed individuals at N2 but at the east coast both undisturbed groups had wider vesicles compared to transplants from north to east ($F_{1,53}$ = 5.40, P = 0.002; post-hoc: P < 0.001) (Figure 3.7).

RGR

Local individuals had greater frond growth than foreign individuals at all sites (Table 3.2A, Figure 3.9). Vesicle growth rates differed between sites and higher performance was found in local individuals at the north coast compared to foreign transplanted individuals (Table 3.2A, Figure 3.9). The average frond growth for undisturbed juveniles at northern sites was 38.56 mm while eastern origin juveniles transplanted to the north coast only grew 19.9 mm on average. Local and foreign individuals performed similarly at E2 and local individuals had less vesicle growth than foreign individuals at E1 (Table 3.2A, Figure 3.9). Here, undisturbed eastern individuals grew on average 52.9 mm and northern origin juveniles transplanted to

the east coast had an average growth of 38.4 mm. For the Home-Away criterion both eastern populations and N2 showed higher RGR (fronds and vesicles) in their local environment compared to their transplanted environment but RGR did not differ between the local and transplanted individuals for N1 (significant Treatment x Population interaction, Table 3.2B, Figure 3.9). Findings were similar for E1 but not N1 when comparing growth rates across the three different habitats (home, same region, different region). For individuals from E1, no changes in RGR were detected when individuals were translocated to a different site in the same region, but RGR was significantly lower when they were transplanted to the north coast (Table 3.2C, Figure 3.9). The performance of individuals from N1 did not change when either translocated to a different site in the same region or to a different region.

Table 3.2: ANOVA comparing relative growth rates of *Hormosira* fronds and vesicles between A) local individuals with transplanted individuals that originated from a foreign environment; B) individuals at their home site with those that were transplanted away; C) individuals at their home site and individuals that were transplanted to a different site in the same region (local) and to a different region (away). Effect size shows the percentage contribution of each factor to the components of variation. Abbreviations: L (local), F (foreign), H (home), A (away).

Source	df	Pseudo-F		effect size	Pairwise test	Pseudo-F		effect size	Pairwise test
A) Local – Foreign									
		variable RGR frond				*variable RGR vesicle*			
Treatment (Tr)	1	**5.01**	**	13.61		**13.88**	***	26.67	
Site (si)	3	14.38		40.55		0.85		0.0	
Tr x Si	3	**5.31**	**	19.96	N1: L > F	**19.36**	***	45.04	N1: L > F
					N2: L > F				N2: L > F
					E1: L > F				E1: L < F
					E2: L > F				E2: L = F
Residual	53			25.88				28.29	
B) Home – Away									
Treatment (Tr)	1	**76.1**	***	41.23		**16.34**	***	17.86	
Population (Po)	3	**3.9**	*	11.43		**23.71**	***	30.66	
Tr x Po	3	**6.27**	***	21.79	N1: H = A	**9.8**	***	26.98	N1: H = A
					N2: H > A				N2: H = A
					E1: H > A				E1: H > A
					E2: H > A				E2: H > A
Residual	53			25.55				24.50	
C) Home – Native – Away									
Treatment (Tr)	2	**10.78**	***	31.58		1.44		5.03	
Population (Po)	1	0.36		0.0		**35.14**	***	36.39	
Tr x Po	2	**5.98**	**	31.86	North:	**9.55**	***	31.24	North:
					H = N = A				H = N < A
					East:				East:
					H = N > A				H = N > A
Residual	37			36.56				27.34	

Bold indicates statistical significance of factor effects (asterisks refer to significance level: *** P < *0.001*, ** P < *0.01*, * P < *0.05*). Sample size was n = 4-12.

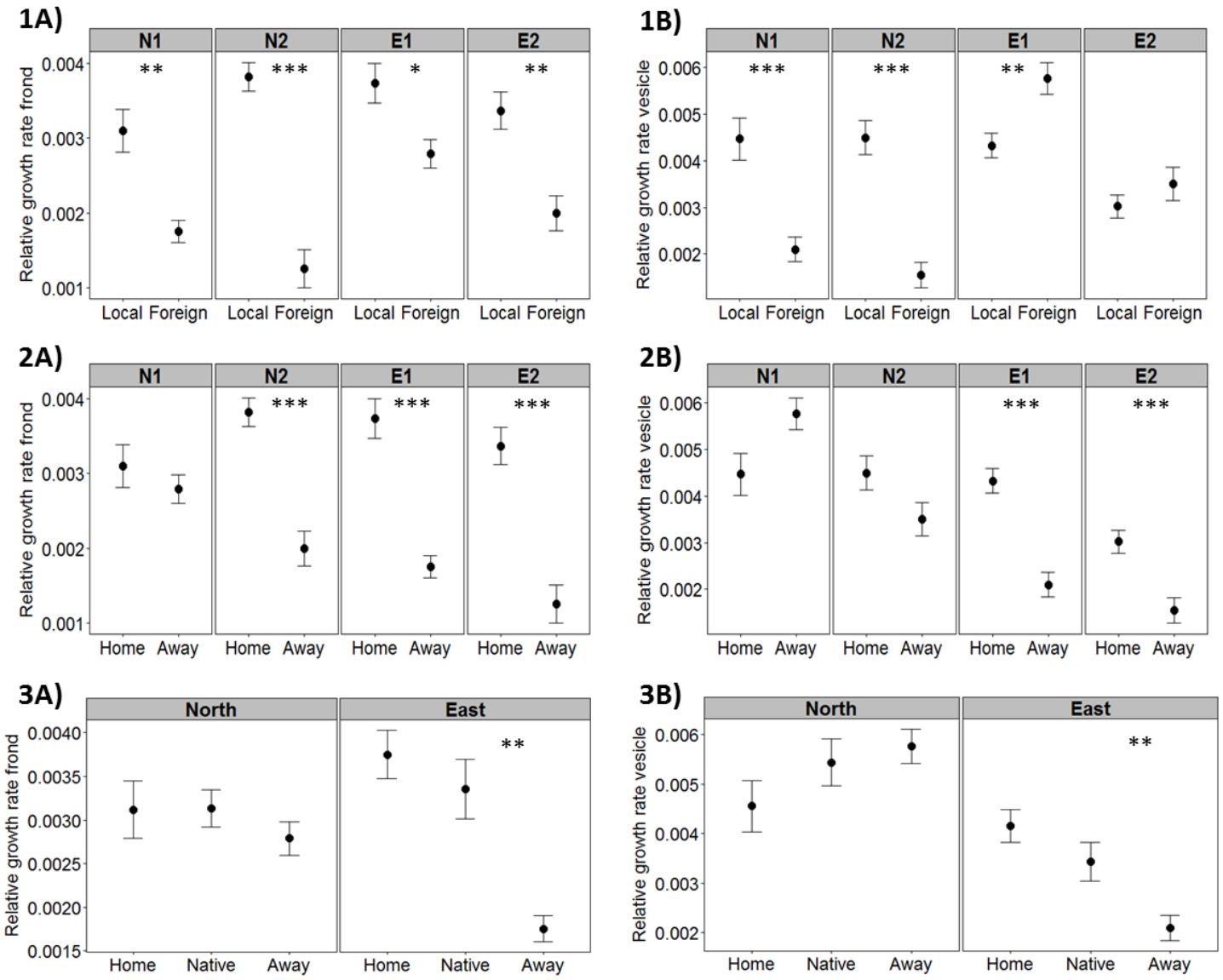

Figure 3.9: Mean relative growth rates (± SE) for A) frond and B) vesicles over the duration of the experiment. Comparisons show 1) the performance of local and foreign populations at each site 2) the performance of each population at their home site and transplanted to a different region (away) and 3) the performance of each ecotype at their local site, when transplanted to a site in the same region (native) and when transplanted to a different region (away). Asterisks refer to significance level: *** P < *0.001*, ** P < *0.01*, * P < *0.05*).

Discussion

Hormosira populations from the different regions showed surprisingly little evidence for a plastic response after transplantation to a new environment despite the different environmental conditions and the potential fitness benefits that are associated with a plastic response (Fowler-Walker et al. 2005). None of the transplanted *Hormosira* juveniles in this study developed an overall morphology similar to undisturbed populations at the recipient site during the experiment and overall morphology remained unchanged in the individuals from N1, N2 and E2 after transplantation. Only individuals from E1 changed their morphology after transplantation to the north coast demonstrating that responses to environmental conditions can be population-specific. Interestingly, individual morphological traits differed in their potential to express plasticity and this depended on the origin of the population. Generally, morphology of individuals of northern origin barely changed when transplanted to the east coast while individuals transplanted from the east coast to the north coast, particularly transplants from E1, grew at a slower rate and displayed shorter fronds and smaller vesicles suggesting that environmental effects were acting on these individuals to provoke a change in these traits.

Under contrasting environmental conditions different pressures drive natural selection in populations and often result in locally adapted populations with phenotypes that have a higher fitness in their local environment than those originating from different environments (Kawecki and Ebert 2004). All local individuals had greater frond growth than foreign individuals but greater versicle growth was only found in local populations from the north coast. Moreover, transplantation of individuals to the foreign site resulted in reduced growth rates compared to local individuals, consistent with local adaptation. However,

individuals transplanted from the north coast to the east coast expressed similar vesicle growth rates to the local population indicating either the potential for northern phenotypes to perform well in the novel environment or that eastern genotypes are less adapted to their native environment.

Environmental factors influencing ecotypic variation

The timing of the tides varied throughout the year at both eastern and north coast sites and strongly contributed to thermal stress encountered by intertidal communities. All *Hormosira* populations experienced longer aerial exposure during summer due to the variability in tides and particularly, the prevalence of extreme low tide events (e.g. spring tides) at this time of the year. Summer low tides usually occurred during the day coinciding with higher air temperatures while winter low tides usually occurred at night or early in the morning and coinciding with low air temperatures. Emersion time was constantly higher for north coast populations resulting in more frequent temperature fluctuations throughout the year. In contrast, populations on the east coast often experienced several days without emersion, particularly during winter. The combination of the semi-diurnal tidal cycle and seasonal variation in the timing of the tides results in longer and more frequent periods of emersion to the air in northern populations, resulting in greater exposure to low temperatures in winter and high temperatures in summer. Typically, exposure to high temperatures is considered stressful for intertidal species but low temperatures can also result in reduced performance in fucoids (Pearson et al. 2000).

During emersion, intertidal seaweeds are exposed to large changes in temperature, solar radiation and intensity, wind and humidity (Helmuth et al. 2011) which all affect thallus temperature and increase risk of desiccation (Beer and Eshel 1983,

Maberly and Madsen 1990). Undisturbed juveniles from both north coast sites were characterised as 'bushy' *Hormosira* morph (see Mueller et al. 2015) with relatively short fronds and small vesicles but high branching. Thallus size is crucial for survival in exposed intertidal environments (Carrington 1990, Gaylord et al. 1994) and a smaller size provides seaweeds with a greater compactness creating a dense canopy (Airoldi 2001) providing shade and protecting organisms in the understory, including juvenile seaweeds, from high temperatures, light and desiccation (Stengel and Dring 1997). Small fronds can merge with the surrounding thalli trapping more water between the fronds (Bell 1995) and reducing temperatures underneath the canopy. Field measurements of dense *Hormosira* canopies on north coast indicate temperature can be > 10° C cooler below the canopy than above it (R. Lewis, unpublished data). While overall extreme and mean temperatures were relatively similar across sites, the greater magnitude and frequency of the temperature fluctuations at northern sites is likely to be important and climatic factors such as spatially varying temperature extremes and ranges can have different ecological consequences (Helmuth et al. 2006, Harley et al. 2012). In addition, intertidal seaweeds often need to be able to repair damaged cell tissue during re-immersion (Davison and Pearson 1996) and this can be particularly important under relatively short immersion times such as those imposed by the semi-diurnal tidal cycle.

Because north coast sites have much longer exposure to air, these *Hormosira* populations often experienced thermal fluctuations in excess of 10 degrees within 24 hours. North coast populations experience more extreme cold or warm weather events. Exposure to the air during summer days can result in temperatures of more than 25˚C beneath the canopy, followed by cool nights or early mornings when temperatures are below 15° C. Higher branching may also be beneficial in helping the seaweed to regulate hydration of the thallus. For example, increased branching

of the thallus is thought to promote connective heat transfer of *Mastocarpus papillatus* leading to warming of the thallus when thallus temperature is below air temperature, and providing evaporative cooling when air temperatures are high (Bell 1995).

In contrast to the north coast, east coast populations had prolonged periods of immersion as not every tidal cycle exposed the experimental plots to air. Most months on the east coast had periods of at least 7 days (usually more) when no *Hormosira* were exposed to air and this could be even longer during storms when swell and waves were high (R. Mueller, personal observation). Temperatures are reasonably constant for a lot of the year, therefore, risk of desiccation, dehydration and thermal stress are less pronounced for east coast *Hormosira,* except during summer. The larger *Hormosira* morph on the east coast may reflect a response to greater submergence time: the intertidal brown seaweeds *Ascophyllum nodosum* (Damant 1937) and *Eregia menziesii* (Chapman 1961) have larger morphs under prolonged immersion. In *Hormosira*, a very large morph occurs in rock pools and estuaries (Ralph et al. 1998, Macinnis-Ng et al. 2005, Bishop et al. 2009) where there would be less emersion. Alternatively, a larger thallus size may provide greater flexibility to resist the strong hydrodynamic forces (e.g. higher wave exposure) on the east coast (Cheshire and Hallam 1988, Bekkby et al. 2014).

Plasticity

Findings showed little indication for plasticity in generating the phenotypic differences in *Hormosira* from the different regions. Nevertheless, similar non-plastic responses have been shown previously in kelps and fucoids after transplantation to different environmental conditions (Blanchette et al. 2002, Roberson and Coyer 2004, Hays 2007). For *Hormosira*, distinct environmental

conditions on the north and east coasts may have selected for phenotypes performing well in their local environment and resulted in different regional ecotypes. However, the absence of plasticity does not necessarily indicate that traits are genetically fixed and several other factors could contribute to the observed patterns. Growth is slow in *Hormosira* and it is possible that the different phenotypes are produced by genotypic variation as a result of developmental canalisation early in the organism's life. Thus, the juveniles in our study may have had their expressed phenotypes already partially or completely determined according to their local site of origin. Although, transplant experiments with *Hormosira* embryos indicate localised adaptation in north coast populations at that early life-cycle stage (i.e. Chapter 4). It could also be argued that the experiment did not go long enough to detect an effect although this is unlikely as plastic responses in other brown seaweeds have been found in much shorter time frames (Fowler-Walker et al. 2005) and on average *Hormosira* juveniles tripled their frond length and increased vesicle number by approximately five times over the experiment.

Some individual morphological traits did show plasticity. Generally, size and vesicle traits appeared to be more plastic than branching related traits but not in all populations. Only juveniles from N1 transplanted to the east coast developed higher overall branching. Interestingly, this was linked to a tendency for more vesicles compared to their native northern individuals and may be due to enhanced growth rates of individuals from N1 under the environmental conditions at E1.

Individuals from both east coast sites developed smaller vesicles when transplanted to the north coast and showed a reduction in frond length when transplanted to the north coast (although the reduction in size in E2 appeared to be at least partially driven by their generally smaller juvenile morphology compared to E1).

Interestingly, the shorter fronds were accompanied by a decline in the total number of vesicles per frond. A reduction in frond length and vesicle size may have allowed the eastern transplants to benefit from increased protection against emersion stress provided by the dense north coast canopy of bushy north coast *Hormosira* populations. Larger fronds would tend to remain on top of the canopy during the outgoing tide, and not gain the same level of canopy protection (Stengel and Dring 1997). Branching remained low for eastern origin transplants indicating that branching traits appeared more fixed but may be linked to future fitness costs. Keeping the thalli short so they can be protected by the canopy may result in a loss of surface area once juveniles grow larger and may ultimately affect fitness in adult individuals. Shorter morphology in transplants can result from increased rates of breakage in fronds under the new hydrodynamic conditions (Blanchette 1997). However, frequent observations during the first 6 months of the experiment did not detect increased breakage among eastern origin *Hormosira* transplanted to the north coast. Furthermore increased breakage would not be expected in plants well adapted to wave forces when transplanted to calmer areas (Blanchette et al. 2002, although see Haring et al. 2002).

Population-level responses

Interestingly, individuals from E1 were the only group that significantly changed overall morphology after transplantation to the other region with frond size and the number of vesicles reduced. Given, that undisturbed juveniles at E1 had larger fronds compared to the other undisturbed populations, their phenotypic appearance changed remarkably under greater levels of emersion stress. This appears to be a population-specific feature and contrasted with the population from E2 which generally had smaller fronds and thus, the need to reduce their size may have been less urgent.

Although plastic responses to the different environmental conditions appeared limited, we did observe variation in morphology between undisturbed juveniles from sites within the same region. This possibly reflected the effect of variable hydrodynamic conditions (Bell and Denny 1994, Wernberg et al. 2003), exposure time or air temperature on small scales (i.e. between sites) (e.g. Williams & Dethier 2005, Hays 2007, Zardi et al. 2013). Temperature fluctuated slightly between sites in each region and was most likely caused by differences in emersion time between sites (see Figure 3.3 and Figure 3.4), although local topographical features may also influence temperature at this scale (Helmuth et al. 2002, Lathlean et al. 2014). Both east coast sites are exposed to large swells although *Hormosira* populations from E2 are more prone to swell due to its location on a headland (Short 2006a) and its higher wave height (Table S1 in appendix). Undisturbed juveniles from of eastern origin differed in their overall morphology, but these differences appear to vanish as adult morphology along the Tasmanian east coast was homogeneous and characterised by elongated fronds and vesicles (Mueller et al. 2015). Smaller fronds at E2 may indicate that juveniles from this population reach their adult morphology later compared to juveniles from E1. We also observed seasonally varying abundances of *Ulva australis* at E1 (but not at E2) peaking during late spring and early summer but declining over summer when temperatures increased (R. Mueller, personal observation) and thus the faster growth and large fronds may enhance competitiveness of juveniles at this site.

Local adaptation

While all local populations showed higher frond growth compared to the foreign populations, only northern populations also showed higher vesicle growth in their local habitat consistent with local adaptation. Both eastern populations had lower frond and vesicle growth when they were transplanted away, although this may be

confounded by their plastic response towards shorter fronds at the north coast environments (e.g. Kawecki & Ebert 2004). The specialisation of a genotype to a particular environment often results in fitness trade-offs in other environments, although experimental evidence shows that adapted populations do not necessarily evolve at the cost of fitness trade-offs (Hereford 2009). Northern populations transplanted to the east coast did not perform worse than the local eastern populations with respect to vesicle growth rates. Given that thallus size in the northern morph appears related to vesicle number (Mueller et al. 2015) this implies that local adaptation to high emersion stress at the north coast is not necessarily linked to loss of fitness in an environment with less emersion, but that these individuals may instead benefit under the different environmental conditions (Reusch 2014), less emersion stress in that case. In addition, while slightly more transplants from the north coast were lost at east coast sites than vice versa (data not shown), we are not confident this indicates differential survival outside of the home site. Estimates of survival can be problematic in transplant experiments such as this and loss of individuals may include both genuine mortality due to the different environmental conditions and the possible loss due to the transplantation method.

Natural selection of phenotypic traits can be advantageous at a population level (Via and Lande 1985, Via 1993) and similar population-specific local adaptation in the fucoid *Silvetia compressa* has been observed in response to transplantation between different intertidal zones (Hays 2007). Local adaptation at small spatial scales (Hays 2007) can also be influenced by a multitude of interacting environmental factors occurring on different spatial scales (Reusch 2014). Local adaptation is usually stronger in populations with restricted gene flow (Knight and Miller 2004). *Hormosira* is a direct developing species with limited dispersal capabilities as

zygotes settle close to their parental generation (McKenzie and Bellgrove 2008). Although fertile fronds are able to float long distances, connectivity between populations along the Eastern Australian Current is low (Coleman et al. 2011b) and would be expected to lead to more locally structured populations (e.g. Zardi et al. 2013). However, the different *Hormosira* ecotypes from the north and east coasts of Tasmania share the same mitochondrial haplotypes (Chapter 5) and thus the underlying genetic basis to the phenotypic variation in *Hormosira* is unresolved until more variable nuclear DNA markers are used to test this idea more strongly.

Conclusion

This study has demonstrated that morphological traits in *Hormosira* vary in their plasticity but individuals do not adjust their overall phenotype to match the phenotype of natal populations following transplantation. Eastern populations had some morphological traits that changed to reflect those of the natal northern populations but northern individuals lacked a plastic response, highlighting the potential for locally adapted northern populations to the higher emersion stress in that region. It remains uncertain whether the lack of plasticity in northern populations is due to genotypic effects or if environmental conditions at the east coast did not trigger a plastic response. Overall, plasticity in *Hormosira* appears to be population specific or weak or, that the phenotype is fixed very early in development. The asymmetrical response to transplantation found in this study highlights the impact of site and population on the phenotype (Benedetti-Cecchi et al. 2006). Generally, populations are adapted to persist and perform well in their home environment (Blanchette et al. 2002) but different populations may respond differently to changes in their local conditions which may affect their resilience. Overall for *Hormosira*, the higher performance in their home environment and the

limited plasticity in response to different environmental conditions suggests constraints exist that may limit a rapid response to environmental change.

Chapter 4

Local adaptation of seaweed embryos to stressful emersion and temperature regimes

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Introduction

Local adaptation occurs when local environmental conditions cause natural selection of advantageous traits resulting in higher fitness of resident genotypes compared to genotypes from other (foreign) habitats (Kawecki and Ebert 2004). There is a rich body of evidence for local adaptation in terrestrial plant species (Leimu and Fischer 2008) but much less for marine species which are assumed to have a lower potential for local adaptation due to high connectivity among populations and high gene flow (Palumbi 1994). However, an increasing number of studies have revealed local adaptation in marine species when a dispersive larval stage is absent or under certain oceanographic conditions that limit gene flow between environments (Dawson 2001, Sherman et al. 2008, Reusch 2014). Although many studies use non-experimental methods (e.g. Sanford & Kelly 2011), transplantation experiments provide a valuable, but underused, approach to identify local adaptation (Sanford and Kelly 2011, Reusch 2014).

Marine intertidal species occur across heterogeneous landscapes where factors such as wave exposure, disturbance, consumer pressure and tidal regimes vary among sites (Benedetti-Cecchi and Trussell 2014). Variation among sites in the timing and duration of emersion at low tide appears important for intertidal species. Typically, sites with long periods of daytime exposure during summer have greater levels of thermal and desiccation stress which results in reduced performance and survivorship (Helmuth et al. 2002, 2006, Williams and Dethier 2005). In intertidal macroalgae, emersion limits nutrient uptake (Davison and Pearson 1996) and reduces photosynthesis (Williams and Dethier 2005), growth (Wright et al. 2004), development (Davison et al. 1993) and recruitment (Taylor and Schiel 2003), while the length of the emersion period affects their ability to recover from desiccation

(Bertness et al. 2006) and cellular damage (Davison and Pearson 1996). Thus populations of macroalgae at sites with long periods of exposure during summer may be locally adapted to these harsh conditions (e.g. Hays 2007, Zardi et al. 2011, 2013).

As a consequence of heterogeneous environments, within-species strategies can evolve in response to differing selection pressures and may result in performance gradients between populations from different habitats (Monro et al. 2007). Hence, a better understanding of the spatial scales of local adaptation will be crucial for species management and predictions on the effects of global change (Sanford and Kelly 2011). For example, if the response to environmental stressors differs between populations as a result of local adaption to spatially variable exposure to those stressors, then climate models that predict shifts in distributions based on the average species response to those stressors are unlikely to be accurate. Furthermore, locally adapted populations might be prioritised during conservation efforts due to their ecological resilience to particular stressors (Sanford and Kelly 2011). It is thus important to understand the mechanisms that drive and maintain differentiation between populations of the same species (Hoffmann and Merilä 1999).

For macroalgae, little is known about whether local adaptation drives within-species variation along environmental gradients, how this affects traits, and translates into fitness. Intertidal seaweeds of the family Fucales are often the dominant habitat-forming species on temperate intertidal shores (Schiel and Foster 2006) and due to their exposed positions on the shore are likely to be subjected to strong trait selection for tolerance of emersion stress (Davison and Pearson 1996, Pearson et al. 2000, Billard et al. 2010). Differences in emersion time is considered to have led to

population differentiation in *Fucus vesiculosus*, with distinct phenotypes occurring in different zones (Zardi et al. 2011) and habitats (Zardi et al. 2013). Moreover, a number of studies have demonstrated dynamic stressors in the intertidal can promote intraspecific morphological variation in fucoids (Blanchette 1997, Wright et al. 2004, Williams and Dethier 2005) highlighting the high potential for population structuring along environmental gradients in intertidal fucoids (Hays 2007). In fucoids, extremely high mortality occurs during the early life-stages (Gunnill 1980, Wright et al. 2004, Dudgeon and Petraitis 2005) which can affect recruitment (Vadas et al. 1992) and have implications for population growth and persistence (Schiel and Foster 2006). Importantly, fucoid embryos are also highly susceptible to thermal and desiccation stress but the presence of a protective adult canopy greatly increases embryo survivorship (Brawley and Johnson 1991, Johnson and Brawley 1998).

Hormosira banksii (Turner) Descaisne (Fucales, Phaeophyceae) is the most common intertidal habitat-forming species in southern Australia (Womersley 1967) and the dominant seaweed structuring intertidal communities in this region (Keough and Quinn 1998, Underwood 1999, Bishop et al. 2009, Hughes et al. 2014). *Hormosira* has a direct-developing life history and gametes have limited dispersal capabilities, with fertilised eggs attaching to the substrate within hours of being released from the parent plant (McKenzie and Bellgrove 2006). *Hormosira* shows a high level of phenotypic variation across its distributional range which has been linked to variable wave exposure and tidal regimes (Ralph et al. 1998, Macinnis-Ng et al. 2005, Mueller et al. 2015). In Tasmania, an island south of mainland Australia, *Hormosira* populations on the north coast have small highly branched fronds with small vesicles compared to east coast populations with elongated fronds and larger vesicles (Mueller et al. 2015). These coasts also differ in their tidal regime, emersion

time and air temperature during low tide with the north coast having a semi-diurnal tidal regime, longer emersion time and more variable temperatures at low tide compared to the east coast which have a mixed-diurnal tidal regime (Chapter 3). Juvenile *Hormosira* originating from east coast populations expressed morphological plasticity in some traits when they were transplanted to the north coast, where they develop a shorter morphology more similar to northern populations. However, no such plasticity was observed when northern-origin populations were transplanted to the east coast (i.e. Chapter 3). In addition, juveniles exhibit higher relative growth rates in their home sites compared to foreign sites. The lack of plasticity and higher home sites performance of northern populations suggest they may be locally adapted to the longer periods of emersion and the resulting increased temperature variability experienced by thalli during emersion. In particular, conditions during summer low tides in the middle of the day when temperatures are warm may be particularly stressful for *Hormosira* and impose strong selective pressures.

Local adaptation appears more common in direct developers (e.g. Sanford & Kelly 2011) and while adaptation to one particular environment increases fitness in that habitat, it can result in reduced fitness of that population in a different environment (Hereford 2009). Here, we explore the importance of local adaptation in embryos from *Hormosira* populations from habitats with contrasting environmental conditions: the north and east coasts of Tasmania. We use a fully reciprocal transplant experiment to assess survivorship and performance of *Hormosira* embryos when transplanted outside their local environments during summer, when thermal stress is highest. These early life-stages are decoupled from developmental effects in a given environment and thus provide a good test of local adaptation (Hays 2007).

Material and Methods

Study species

Hormosira individuals occur as one or several fronds arising from a single holdfast with the fronds made up of a chain of water-filled vesicles that vary in size and shape (Womersley 1967). *Hormosira* is perennial and dioecious with gametes contained in conceptacles and released during low tide providing year-round recruitment (Bergquist 1959). Fertilisation occurs externally and the direct-developing zygotes settle close to their parental generation (McKenzie and Bellgrove 2006) resulting in limited dispersal capabilities and low population connectivity (Coleman et al. 2011a). *Hormosira* often attain high population densities and form dense intertidal canopies (Clayton 1984).

Study sites

This study was conducted in Tasmania, Australia in January 2015. We focused on populations from the Tasmanian north and east coast because these regions have distinct *Hormosira* ecotypes: small thalli with increased branching at the north coast; elongated thalli with larger vesicles at the east coast (see Chapter 2; Mueller et al. 2015). *Hormosira* populations in this study were from Beechford (N1; 41°01'22"S, 146°56'39"E) and Bell Buoy Beach (N2; 41°02'23"S, 146°49'56"E) on the north coast and at Sloop Reef (E1; 41°12'17"S, 148°16'53"E) and Four Mile Creek (E2; 41°33'26"S, 148°17'35"E) on the east coast (these were the same sites that were used in Chapter 3). At all of these sites *Hormosira* is very abundant in the intertidal and forms dense beds of up to 100% cover. The substratum at north coast locations is characterised by cobble-basalt rock-flats while granite boulders dominate the east coast sites (Short 2006a). Although all locations shared a

moderate degree of wave exposure, wave dynamics are generally more pronounced on the east coast (Short 2006a).

Tidal dynamics

The semi-diurnal tides (two tidal cycles within a 24 hour period) of north coast result in higher tidal amplitudes (McInnes et al. 2011) and more frequent emersion and greater temperature fluctuations experienced by *Hormosira* (Chapter 3). In contrast, the mixed diurnal tides of the east coast result in small fluctuations in the height of the high and low tide at certain times of the year (see Australian Hydrographic Service 2015) causing prolonged periods of submersion for *Hormosira*. Likewise, the time of the day at which the lowest low tide and highest high tide occur varies predictably throughout the year. Both coasts experience the lowest low tide during winter (May-Sep), usually at night or early in the morning. The lowest low tide during summer (November – March) usually occurs around midday or mid-afternoon, often as extreme lows during perigean spring tides (Australian Hydrographic Service 2013). Although both coasts experience midday and afternoon exposure during summer when high temperatures are more likely, the north coast populations are exposed to more frequent and longer cumulative emersion periods and consequently experience greater amplitude of daily fluctuations in temperature (i.e. Chapter 3).

Temperature and emersion times

Temperature was recorded every 10 minutes at sites using HOBO ® TidbiT v2 data loggers (Onset computer corporation, Bourne, MA, USA). The temperature monitoring was part of a larger study determining thermal fluctuations and extreme temperatures at these sites over a year (April 2014 - April 2015). Two loggers were deployed under the adult *Hormosira* canopy at each site and mean temperature

profiles across the four loggers for each region were plotted for the month of January 2015 when the experiment was conducted. We also estimated the average daily maximum (ADM) temperature defined as the average peak temperature across all days of January 2015 as a measure of habitual high temperatures encountered by organisms (see Helmuth et al. 2002). Emersion time per day was numerically estimated as the duration in which experimental plots were exposed to air. This was determined by integrating 10 min time intervals for which the forecasted chart datum (tidal height) correlated to exposed individuals (i.e. their relative height at each site). The total emersion time was calculated by summing daily emersion time for all days of January 2015.

Reciprocal transplant experiment

We conducted the reciprocal transplant experiment during January 2015 when spring tides caused extreme low tides during the day that coincided with high summer temperatures creating periods of high thermal and desiccation stress. Embryos were harvested from each of the two populations from each region (north: BF and BB; east: SR and FM) and reciprocally transplanted among sites where each site was a recipient for all four populations (replicated by site in each region).

Culturing of embryos

Mature thalli were collected randomly along a 15 metre transect placed at mean sea level height at each site in early January 2015. Given the limited dispersal range of *Hormosira* we sampled thalli separated by a minimum distance of 50 cm from each other to minimise confounding by family effects. Individual fronds were placed in seawater-filled Ziploc-bags, transported to the laboratory and stored overnight at 4˚C. On the next day tissue was cleaned in a Betadine solution (1ml/L filtered seawater) for 30 seconds before individual frond parts (5-7 vesicles) were placed

into individual petri dishes and exposed to 30°C filtered seawater to induce gamete expulsion. Gametes were released immediately, and fronds were removed from petri dishes after 5 minutes. Gametes can be identified as female (herein called eggs) due to their green colour, or male (herein called sperm) due to their orange colour (McKenzie and Bellgrove 2008).

Sex of gametes released from individual thalli was determined and then male and female gametes pooled in separate 200ml jars. Eggs from the mixed egg solution (pooled across ten females per population) were then pipetted onto plain ceramic tiles (2 x 2 cm; ceramic face up, n = 180 tiles) placed in plastic jars (eight tiles per jar) filled with 150 ml of filtered seawater before aliquots of the mixed sperm stock solution (pooled across ten males from the same population) were added to the tiles. We targeted successful settlement densities of approximately 500 eggs per cm^2 as this is a good proxy for settlement in the field (Taylor and Schiel 2003). Tiles were left undisturbed for two hours to allow secure attachment of zygotes and then transferred to clean plastic 500 ml containers with 300 ml of filtered seawater(Clarke and Womersley 1981). The following day tiles were cleaned of residual unfertilised material by gently agitating the submersed tiles, and moved to new containers (500 ml size filled with 300 ml filtered seawater). Containers were incubated at 17°C under a 14h light: 8h dark cycle at >100 µmol photon m^{-2} s^{-1} for six more days. Mean settlement densities (± SE) were 404 ± 24 embryos per cm^2.

Out-planting of embryos

Secure adhesion to the substrate is crucial for survival of *Hormosira* embryos in the field (Taylor and Schiel 2003) and older embryos have a better chances of survival in transplant experiments (three weeks vs three days) (Dunmore 2006). Embryos were cultured for one week before out-planting to allow secure attachment and

improved survival. A longer culture period may have improved survival however, we aimed to minimise the effects of embryo acclimatisation to lab conditions. All parental plants were collected from field sites one day prior to fertilisation and all embryos were cultured under the same conditions for 7 days in the laboratory before out-planting to the appropriate region. Embryos for out-planting to the eastern sites were fertilised on 09/01/2015 and out-planted on 16/01/2015; embryos for out-planting to the northern sites were fertilised on 16/01/2015 out-planted on 23/01/2015.

Before out-planting, ceramic tiles were rinsed again to remove zygotes that were not securely attached and then glued to a 5 cm x 7 cm x 0.45 cm thick fibre cement base plate (Hardiflex, Australia) using underwater epoxy (A-788 Splash Zone Compound). One tile from each of the four populations was attached to the each base plate. In the field, the base plate was fixed by retainer clips to a supporting anchor structure consisting of a larger fibre cement plate (8 cm x 7 cm) and this plate was glued to the rock substrate with underwater epoxy. Finally, the entire 8 cm x 7 cm experimental unit was caged with plastic mesh (grid size 7 mm x 7 mm, Whites Wires, Australia) to exclude grazers and reduce scour from the nearby canopy. This design allowed the base plate and embryo tiles to be rapidly unclipped and returned following non-destructive sampling via photography of embryo growth and survivorship. Ten units containing one tile each from the four populations were placed under the adult canopy at all four sites. Tiles were out-planted to northern and eastern sites one week apart to allow sequential sampling at the four sites, taking account of travel time and low tides in the different regions.

Embryo performance and survivorship

Fucoid embryos can be easily identified using low power magnification (Brawley and Johnson 1991, Schiel and Foster 2006). After seven days of culturing under laboratory conditions and immediately prior to out-planting we took separate photos of each tile (Olympus TG2, F2.0-4.9 25-100mm) after they were glued onto the base plate. We also measured the size of n = 5 embryos for each tile under a portable digital microscope (Dino-Lite, AM4113; x 50 magnification). After initial photography, base plates were then stored in small containers lined with damp paper towel and transported to the sites. Travel times were arranged so that embryos went into the experimental units at each site just before the incoming tide to avoid unnecessary prolonged exposure to the air. Mortality during transportation was assessed with tiles that were transported to the sites under the same conditions as the out-planted tiles. All embryos survived the trip to the sites. *In situ* measurements of embryos were intended to be taken after 2 days, 4 days, and 30 days post transplantation. However, survival of embryos was very low after 30 days and tiles were profoundly overgrown in the field making it difficult to accurately estimate embryo density and size. Furthermore, data collected 2 days and 4 days post-transplantation showed similar trends and hence only data from the 4 day measurements are presented in this study. At each measurement interval base plates were removed from the caged units when exposed at low tide and photos of density and embryo size taken. Individual tiles were photographed with the same two cameras used for the initial assessment. This allowed us to assess all the tiles at each site during the short workable low tide intervals and count embryos from tile photos at a later time. Plates were returned to the experimental units immediately after photography and always before the incoming tide. Survivorship after 4 days was calculated as a percentage of the initial number on each tile. Performance was

measured as the relative growth rate (RGR) after 4 days and calculated based on the mean embryo length per tile as [ln (final measurement) – ln (initial measurement)]/number of days (Evans 1972).

Variation in egg size

We also measured egg size among populations to account for the possibility that egg size may influence embryo fitness (Clark et al. 2013). Immediately after release of gametes we measured the diameter of 20 randomly selected eggs from 10 female *Hormosira* for each site with a portable digital microscope (Dino-Lite, AM4113; x 50 magnification).

Statistical analyses

Variation in egg size was tested with a three-factor hierarchical ANOVA with Female (random) with 10 levels (1-10 individuals) nested within Site (random) with two levels (N1 and N2; E1 and E2 respectively) nested within Region (fixed) with two levels (north, east). Only a limited number of replicates could be managed at each site during any given low tide, therefore the transplant experiment was designed to assess performance of each population (i.e. from each site) based on the broad environmental differences between regions (i.e. different tidal regimes emersion time and air temperature during emersion). Our design aimed to test whether populations perform better in their home regions than when transplanted to a different region, and whether populations in their local region perform better than foreign populations that originated from a different region. Survivorship and growth rate of embryos between populations and destinations (i.e. transplanted regions) were compared with a three factor mixed model ANOVA with the factors Population (fixed) with four levels (N1, N2, E1, and E2), Destination (fixed) with two levels (north, east) and Block (random) which was nested within the Population x

Destination interaction. Tukey HSD posthoc tests were used to explore significant factor effects. Data was checked for homogeneity of variances and normality by visually inspecting residual plots and data was transformed if required. All analyses were carried out using the R (v. 2.15.1) software package.

Results

Temperature and emersion time

Average daily maximum temperature during January 2015 was higher at northern sites (ADM north: 22.45˚C) compared to eastern sites (ADM east: 19.27 ˚C) confirming the generally higher temperature peaks at the north coast (Figure 4.1). Air temperatures on the north coast reached > 5° C above the average ocean temperature on 10-12 days compared to approximately 4 days on the east coast (Figure 4.1). Moreover, the diurnal tides on the north coast resulted in larger temperature variation up to almost 15˚C within 24 hrs on a single day reflecting exposure to the air during both the day and night (Figure 4.1). In contrast, temperature curves from the east coast fluctuated less with periods of several consecutive days when the temperature did not vary much (January 9-10 and January 12-15) due to no emersion on several days (e.g. January 13-15, Figure 4.1) and presumably prolonged submergence caused by the local meteorology (e.g. swell or storms; e.g. January 9-10).

Temperature profiles during the experimental periods reflected the overall monthly patterns reasonably well. During the experiment on the east coast temperatures only fluctuated slightly with a tendency to slightly colder than average temperature due to the local meteorological conditions. The only exception was day 4 post-transplantation when temperatures beneath the canopy peaked at almost 25 ˚C for

around 1 hour, although photos were taken earlier in the emersion cycle (Figure 4.1). The timing of the experiment on the north coast coincided with both relatively cooler and warmer temperatures on day 3 and 4 post-transplantation which were caused by cool temperatures during emersion at night or early morning and warm temperatures during daytime emersion. On day 4 post-transplantation, temperatures remained above 25 ˚C for 2.5 hours and measurements were taken approximately half way through this period.

Although the lowest low tide in January always occurred during daylight, tidal patterns varied greatly between regions. Overall in January, estimated exposure time varied between the coasts with longer exposure on the north coast (total exposure time: 121:15 hrs) compared to the east coast (74:05 hrs) reflecting a second period of emersion on most days. The days during the out-plantings had relatively long emersion times for the month for the respective experiments and the exposure time for the north coast experiment was the highest across the month with over 5 hours exposure on each day (Figure 4.1).

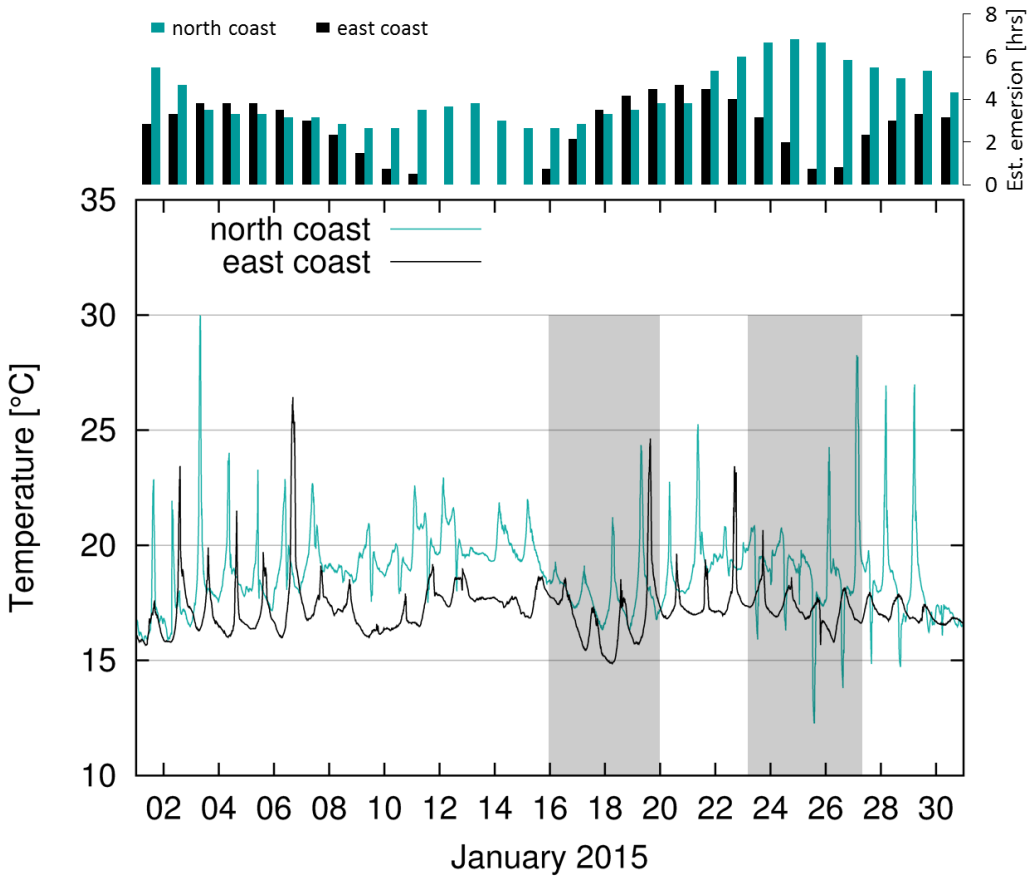

Figure 4.1: Estimated emersion times (A) and temperature time series (B) pooled across sites within north and east coasts during January 2015. Estimated emersion shows daily time (hrs) *Hormosira* embryos were exposed to air (north coast emersion is shown as blue bars and east coast emersion is shown as black bars). For temperature, each line represents data for one region averaged from four data loggers (two per site) that were attached under the canopy. Grey shaded areas indicate days during which experiments were conducted at the east (January 16 – 20, 2015) and north coasts (January 23 – 27, 2015).

Reciprocal transplant experiment

Survivorship

Overall survivorship was highest (approximately 70%) in both northern populations (N1, N2) at their local north coast destination and contrasted to the two eastern populations (E1, E2) which had only 41-45% survivorship at their local eastern

destination (Figure 4.2). The effect of destination on the survivorship of *Hormosira* embryos strongly depended on the origin of the populations (significant Population x Destination interaction; Table 4.1). Embryos from both northern populations had higher survival (16-23%) when they were out-planted to their local north coast habitat compared to when they were out-planted to the east coast (Table 4.1, Figure 4.2). In contrast, the survivorship of embryos originating from the two eastern populations E1 and E2 did not differ between the east coast (i.e. their local habitat) and the north coast (Table 4.1, Figure 4.2). Despite the reduced embryo survivorship of both northern populations in their foreign environment, there was no difference in survivorship among the four out-planted populations N1, N2, E1, and E2 on the east coast (average survivorship between 41-56%). On the north coast, both northern populations had much greater survivorship than the two eastern populations (30-45% higher) with survivorship particularly low in the population from E1.

Table 4.1: ANOVA testing for differences in survival and Relative Growth Rate (RGR) between the population embryos were transplanted from (levels: N1, N2, E1, E2) and the destination embryos were transplanted to (levels: north, east). Effect size shows the percentage contribution of each factor to overall variation.

Subject	**df**	**MS**	**F**	**p**	**Effect (%)**	**MS**	**F**	**p**	**Effect (%)**
		Variable survival				*Variable RGR*			
Transformation		Square-root				Fourth-root			
Population (Pop)	3	34.46	16.49	**<0.001**	25.49	0.004	3.76	**0.015**	11.06
Destination (D)	1	8.33	3.99	0.05	7.91	0.027	29.09	**<0.001**	24.94
Pop x D	3	13.79	6.60	**<0.001**	21.68	0.002	2.41	0.08	11.17
Block (Pop x D)	72	2.09	0.83	0.78	0.00	0.001	0.64	0.97	0.0
Residuals	80	2.51			44.92	0.001			52.83
Tukey HSD									
Pop		N1 = N2 > E1 = E2				E1 > N1 = N2 = E2			
D						north > east			
Pop x D - level Pop		N1: north > east; N2: north >east; E1: north =east; E2: north = east							
Pop x D - level D		north	N1=N2, N1>E1, N1>E2, N2>E1, N2>E2, E2>E1						
		east	N1 = N2 = E1 = E2						

Bold indicates statistical significance of factor effects.

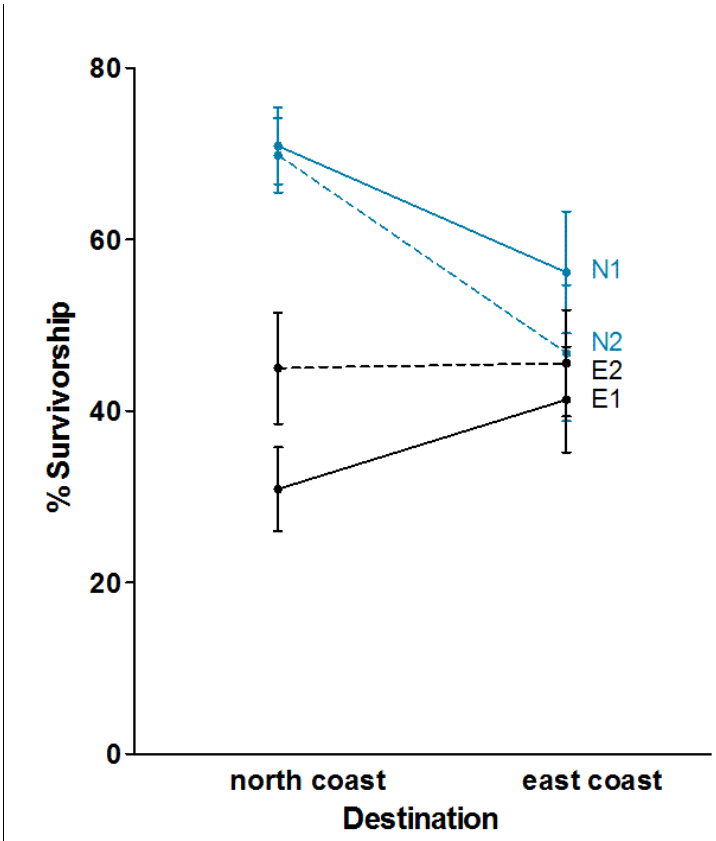

Figure 4.2: Mean (± SE) percentage survivorship of *Hormosira* embryos 4 days post-transplantation for each population transplanted to the north and east coasts. Each bar represents n=20 tiles pooled across sites in each destination region. Site abbreviations are N1 (Beechford; north coast), N2 (Bell Buoy Beach; north coast), E1 (Sloop Reef; east coast) and E2 (Four Mile Creek; east coast).

RGR

Destination had a strong effect on RGR of *Hormosira* embryos. All populations exhibited higher growth rates at northern sites which accounted for almost 25% of the total variation (Table 4.1, Figure 4.3). Growth rates also varied among populations. Embryos from E1 showed higher growth rates than embryos from other populations out-planted to each destination (Table 4.1). Residual variation was high and contributed to more than 50% of the total variation in growth (Table 4.1). There was a trend of faster growth in northern embryos when out-planted to

their local north coast habitat compared to the east coast habitats (Figure 4.3), although this was not significant (Population x Destination interaction P = 0.08).

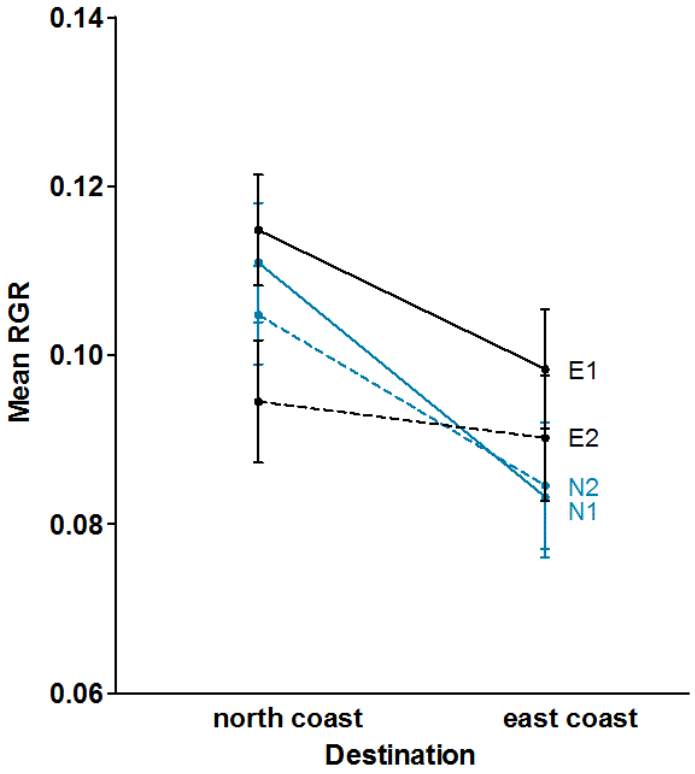

Figure 4.3: Mean (± SE) relative growth rate (RGR; the increase in embryo size per day) of *Hormosira* embryos 4 days post-transplantation for each population transplanted to the north and east coasts. Each bar represents n=20 tiles pooled across sites in each destination region. Site abbreviations as for Figure 4.2.

Variation in egg size

Findings of the reciprocal transplant experiment were unlikely to be influenced by egg size among sites (P= 0.13) or regions (P = 0.7). Instead egg size varied greatly among females within sites (P < 0.001) highlighting the individual-level variation within *Hormosira* populations.

Discussion

This study has demonstrated that *Hormosira* embryos originating from the north coast of Tasmania had higher survivorship in their local environment than when they were transplanted to the east coast. Moreover, at the north coast destination, embryos originating from local northern populations had much higher survivorship than embryos that originated from the east coast. In contrast, the survivorship of embryos from eastern populations did not differ between their local and foreign environments. Generally, growth rates were higher when embryos were out-planted at the north coast implying that eastern origin embryos experienced enhanced growth in the foreign environment. The performance of both northern populations is highly indicative of local adaptation (Kawecki and Ebert 2004, Hereford 2009) resulting from different selection pressures acting on northern populations.

Although largely understudied, complex patterns of local adaptation in seaweeds have been previously demonstrated in response to a variety of environmental selection gradients including salinity (Serrão et al. 1996a, Pearson et al. 2000), latitude (Araújo et al. 2011) and across zones (Hays 2007), highlighting the role of evolutionary processes in structuring populations that live under various environmental conditions (Hoffmann and Merilä 1999, Sanford and Kelly 2011). Also, *Hormosira* showed considerable spatial differentiation and the observed differences in performance between north and east coasts embryos appear to reflect the broad environmental differences between the coasts.

The semi-diurnal tides on the north coast of Tasmania result in longer and more frequent emersion periods and thus subject intertidal species to increased levels of emersion stress (e.g. Zardi et al. 2011). Emersion stress can severely affect seaweed recruitment and particularly, the early life-stages are susceptible to desiccation

(Brawley and Johnson 1991, Davison et al. 1993, Johnson and Brawley 1998, Hays 2007). Thus, the greater amount of time northern *Hormosira* are exposed to air represents a key environmental stressor acting on the embryos (Chapter 3). Desiccated adult thalli are affected by nutrient limitation (Davison and Pearson 1996) reducing photosynthetic rates and determine recovery time (Williams and Dethier 2005). Our study indicates that the same factors may influence embryos. Greater thermal fluctuations at the north coast resulted in several occasions when exposure on a warm summer day created temperatures of more than 25˚C under the canopy, although a second exposure followed with relatively cool temperature during the night (Figure 4.1). Consequently, well adapted northern populations may have a higher tolerance to emersion stress and require less time to recover after emersion resulting in higher fitness at their local habitat.

A complex canopy formed by highly-branched intertidal seaweed populations often provides protection from desiccation (e.g. Stengel & Dring 1997) and thermal stress (e.g. Bell 1995) while further safeguarding recruits from the harsh physical conditions during aerial exposure (Brawley and Johnson 1991, Johnson and Brawley 1998, Lamote et al. 2007). *Hormosira* populations from the north coast are characterised by highly branched, but relatively short fronds which merge nicely with the surrounding fronds to form a dense canopy (Mueller et al. 2015). This 'bushy' phenotype did not change when northern *Hormosira* juveniles were transplanted to the east coast, indicating fixed morphological traits and thus reflecting the high potential for localised adaptations in those populations, possibly in response to the greater emersion stress at the north coast (i.e. Chapter 3). Findings from this study further support the habitat-specific patterns of local adaptation but importantly, demonstrate that effects are present very early during the organism's development.

Mortality in fucoids is very high and often depends on environmental conditions (Schiel 2004, Wright et al. 2004, Schiel and Foster 2006) and as result only individuals capable of resisting the harsh physical forces can contribute to the gene pool of future generations (Coelho et al. 2000). Selection for emersion tolerance has been found in embryos of the intertidal fucoid *Silvetia compressa* which showed distinct adaptations to their parent's local emersion regimes (Hays 2007). Given that survival of early life stages is crucial for the persistence of local populations (Vadas et al. 1992, Coelho et al. 2000), the higher emersion resilience possibly forms an important strategy in northern origin *Hormosira* populations which is effective immediately post-fertilisation.

Despite reduced emersion stressors on the east coast the northern origin embryos showed lower survival and growth in this environment, suggesting that adaptation to the north coast regime may be linked to physiological cost in a different environment (Van Tienderen 1991, Dethier et al. 2005). Selection for specialised phenotypes with high performance in their local environment can lead to lower genetic variability in locally adapted populations (Hoffmann and Parsons 1993, Uller et al. 2002), which in turn may reduce the potential for plasticity and performance under changing environmental conditions (Eads et al. 2012). However, survival of embryos from both northern sites out-planted to the east coast was similar to eastern embryos in their local environment. While *Hormosira* is less frequently exposed to air on the east coast, there is higher wave energy at those sites (Short 2006a). Wave exposure can have profound effects on seaweed morphology and physiology (Blanchette 1997, Roberson and Coyer 2004, Fowler-Walker et al. 2005) and furthermore represents one of the crucial variables determining the survivorship of early life stages of seaweeds (Vadas et al. 1992). In *Hormosira*, the risk of embryo dislodgement increases with increasing wave exposure (Taylor and

Schiel 2003). Our findings indicate that locally adapted northern embryos are not disadvantaged compared to the native embryos in the foreign environment where wave exposure is likely to be higher and thus emphasise that local adaptation is not always associated with fitness traits trade–offs (e.g. Hereford 2009).

In contrast to the north coast embryos, no fitness loss was observed in embryos of eastern origin when transplanted to the northern habitat. Emersion time and associated temperature stress is lower and more predictable on the east coast, however other stressors exist for *Hormosira*. Prolonged submergence can lead to reduced light intensity which can affect the performance of fucoid embryos (Irving et al. 2009) while higher wave energy increase mortality and dislodgment in recruits (Vadas et al. 1992). It is thus possible that eastern individuals are less specialised but have greater plasticity through an ability to tolerate a wider range of environmental variation (Van Tienderen 1991). Plasticity is demonstrated by the potential to develop phenotypes with high performance in several environments (Franks and Hoffmann 2012) allowing adjustment to a range of environmental conditions (Dudley and Schmitt 1996, Schlichting and Pigliucci 1998, Agrawal 2001). As a result, eastern origin embryos were capable of tolerating the novel conditions at the north coast suggesting they represent generalist phenotypes in the environment. Furthermore transplant experiments with *Hormosira* juveniles indicated a plastic response by eastern origin individuals, which developed reduced frond and vesicles size when transplanted to the north coast, possibly allowing them to access the dense canopy protection in those habitats (Chapter 3).

Embryos from E1 had the lowest survival but the highest growth at both destinations and surprisingly growth was highest in the foreign habitat (i.e. north coast). Moreover, we observed a considerable amount of residual variation within

populations which suggested that tolerance may vary among individuals (Reusch 2014). Demes et al. (2013) noted a trade-off between survival and growth in the brown seaweed *Egregia menziesii* where high growth rates were obtained at the cost of survival to guarantee performance. Presumably only those genotypes from E1 with the prerequisites to tolerate the novel conditions benefit under the north coast regime. Nevertheless, it remains unclear whether this is caused by a high-risk boost in performance where all energy is invested into growth, or due to the environmental conditions at the north coast being more favourable for embryos from E1. The latter seems less likely as survival was slightly reduced at the northern location compared to the local east coast environment.

The northern Tasmanian coast borders the shallow water basin Bass Strait that separates mainland Australia from Tasmania. The basin sits on the continental shelf and the north Tasmanian shoreline is generally characterised by a relatively flat coastal slope (Short 2006a). Due to the local bathymetry near-shore sea surface temperatures show distinct inter-annual variability with a rise in summer and a drop in winter respectively (Sandery and Kämpf 2007). During January 2015, the average temperature was higher at north coast compared to the east coast with the north coast profile usually well above the east coast indicating not only warmer air temperature but also warmer water temperature (see Figure 4.1). Increasing water temperature resulted in increased growth in the embryos of the fucoids *Fucus evanescens* (Major & Davison 2010) and *Pelvetia fastigiata* (Davison et al. 1993). Thus, the warmer water on the north coast may be the reason for the increased growth in out-planted embryos regardless of their origin. In addition, the dense canopy created by *Hormosira* on the north coast may further contribute to the elevated growth patterns due to its ability to efficiently reduce the high temperature stress under the canopy (R. Lewis – unpublished data). Nevertheless, as

experimental plots in this study were caged, therefore the survival and growth rates were obtained in the absence of both scour and grazing, both of which can impact recruitment and post-recruitment mortality in seaweed populations (e.g. Vadas et al. 1992).

We have previously shown that juvenile *Hormosira* from the site E1 have larger fronds compared to juveniles from E2 (i.e. Chapter 3) although no size differences were detected in adult thalli between this and other east coast sites (Mueller et al. 2015). We observed high densities of *Ulva australis* in between the *Hormosira* canopy during spring and early summer which declined later in the season when temperatures peaked (R. Mueller – personal observation). Increased growth rates in the population from E1 may be a key component for recruitment of *Hormosira* during times when competition is high.

Hormosira generally release gametes with each incoming tide (Gunthorpe et al. 1995) although early release at low tide is reported (Taylor & Schiel 2003, Bellgrove et al. 2010). However, prolonged submergence either caused by persistent swell or variability in tidal height can inhibit gamete release (Serrão et al. 1996b). Two prolonged submergence events of between 2 and 4 days were recorded during January 2015 but we have also observed longer submergence periods during the same year (i.e. Chapter 3). In *Fucus vesiculosus* delayed gamete release results in too many mature gametes within conceptacles and changes the timing of the release from high to low tide such that gametes were released at less favourable conditions (Berndt et al. 2002), affecting fertilisation success (Pearson and Brawley 1996, Serrão et al. 1996b) and potentially gamete fitness. Theoretically, a certain extent of tolerance to prolonged immersion would be expected in gametes from eastern populations in order to match the high potential of a delayed gamete exudation.

While maternal environment effects were detected in the size of female gametes, this variation was largely due to within population and within individual variability. Interestingly, we observed more gametes in individuals from E2 (R. Mueller – personal observation; both batches) which also has the highest wave energy among our sites (Short 2006a). Environments with high water motion have an increased risk of gamete dilution which can lead to reduced fertilisation success (Serrão et al. 1996b). Thus, the observed higher gamete abundances in *Hormosira* from E2 may reflect a strategy to cope with the higher turbulences in this environment and increase the chances for successful recruitment. Although a higher gamete release may come at a higher photosynthetic cost for the organism (Serrão et al. 1996b), this does not seem to translate into future performance consequences as rates for survival and growth for embryos from E2 were similar between northern and eastern destinations and were comparable to the performance of the other eastern population (E1) in their local environment.

Although micro-environment variation can affect performance and adaptation in fucoids (Hays 2007) similar direction and magnitudes of change in both northern population performance (Figure 4.2 & Figure 4.3) is consistent with adaptation to overall environmental conditions of the north coast. Embryo survival is a crucial step in an organism's life history. While adaptive forces may differ at other development stages, our findings present strong evidence for local adaptation by *Hormosira* populations to long emersion time and increased temperature variation experienced on the north coast. This in turn appears to result in spatially different patterns of local adaptation in *Hormosira*.

Chapter 5

Historical and contemporary processes affecting genetic variation in the widespread intertidal seaweed *Hormosira banksii*

This chapter has been submitted for publication and the preliminary citation is:

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Introduction

The distribution of marine benthic species and patterns of connectivity among populations are driven by a combination of historic and contemporary oceanographic processes (Avise 1996, Palumbi 1996) although the relative contribution varies between species due to differences in life-history and biology (Avise 1996, Sivasundar and Palumbi 2010, Luiz et al. 2012, Miller et al. 2013). Moreover, while habitat availability and continuity enhance gene flow (Ayre et al. 2009, Alberto et al. 2010), disruption to dispersal due to unsuitable habitat can limit recruitment (Billot et al. 2003, Fraser et al. 2010a), reduce gene-flow and increase genetic structuring of populations (Li et al. 2013). It is thus crucial to determine whether populations are panmictic (open) or isolated (closed) to enhance the understanding of species' ecology and improve species' management and conservation (Coleman et al. 2009).

In southern Australia, the most recent palaeoclimatic changes occurred during the final stage of the Pleistocene epoch (last 100,000 years) and were characterised by substantial fluctuations in sea levels, water and air temperature, and sea ice cover (Lewis et al. 2013). In particular, low sea levels during the last glacial maximum (LGM), approximately 25, 000 years ago, connected the present day island of Tasmania to mainland Australia (Lambeck and Chappell 2001) forming a physical barrier to east-west gene flow for many marine benthic organisms including seaweeds (Fraser et al. 2009, Durrant et al. 2015), invertebrates (Waters and Roy 2003, Dawson 2005, York et al. 2008, Ayre et al. 2009, Li et al. 2013) and vertebrates (Charlton-Robb et al. 2014, Moore and Chaplin 2014).

Boundary currents are the main factor facilitating gene flow and connectivity for marine species in southeastern Australia (Hunt and Ayre 1989, Banks et al. 2007,

Sherman et al. 2008, York et al. 2008, Coleman et al. 2011b). The dominant currents affecting this region are the Eastern Australian Current (EAC) which flows down the east coast of Australia, including Tasmania, and the Zeehan Current (ZC) which is an extension of the Leeuwin Current and flows from western Victoria down the west coast of Tasmania (Figure 5.1). Both currents have a southward directed flow with a clear seasonality with the EAC typically strongest in summer and the ZC typically strongest in winter (Ridgway 2007). However, patterns of genetic structure do not always match the patterns of these currents (Sherman et al. 2008) due to long stretches of unsuitable habitat that create contemporary barriers to dispersal and gene flow (Coleman et al. 2009, Fraser et al. 2010a). On the southeastern coastline of mainland Australia, Ninety Mile Beach spans 300 km of sandy shore and results in genetic differentiation across the barrier for several rocky shore species (Hunt 1993, Billingham and Ayre 1996, Waters et al. 2005, Hidas et al. 2007). In this region, three distinct biogeographic provinces have been recognised based on differences in intertidal species composition (Peronia (east), Flindersia (west) and Maugea (south-east) (Waters et al., 2010, *sensu* Bennett & Pope, 1953). A combination of complex hydrodynamic patterns, temperature gradients and historic and contemporary disjunctions delineate the boundaries between these biogeographic provinces (Miller et al. 2013) and help define biogeographic structures. Increasing evidence demonstrates that the location of major barrier systems and phylogenetic breaks corresponds to the prominent province boundaries (Waters and Roy 2003) and that genetic population structuring varies less within provinces than between provinces (Li et al. 2013).

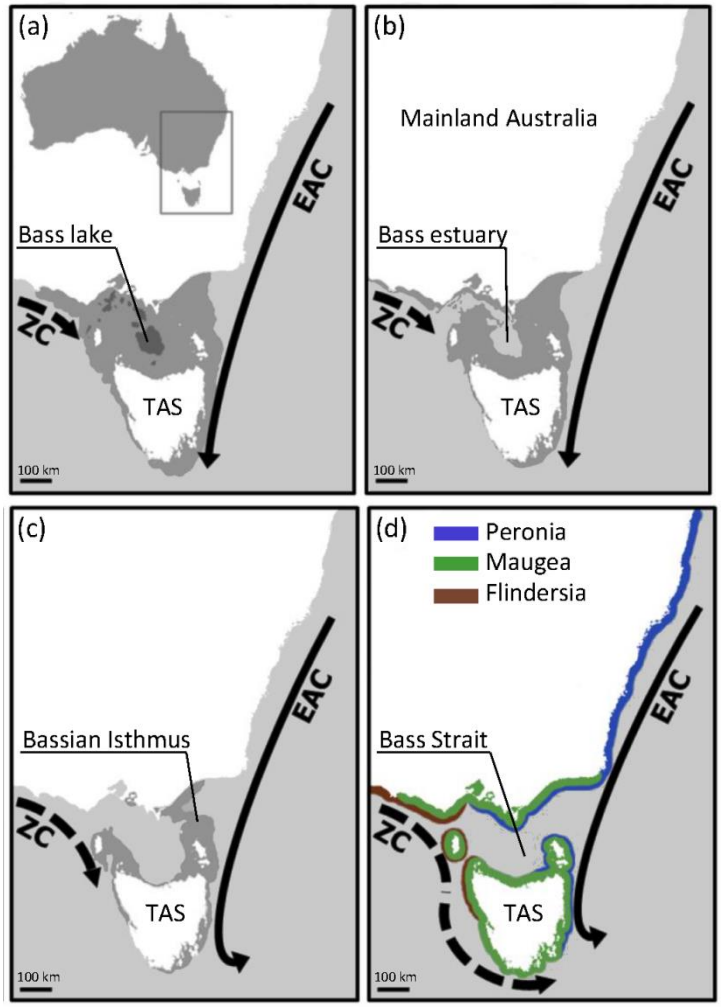

Figure 5.1: Approximate shoreline for Bass Strait between mainland Australia and Tasmania with grey shaded areas showing four different sea levels during recent geological history (reconstructions modified from Lambeck and Campbell (2001)). A) During the last glacial maximum (LGM) 25000 years ago when Tasmania was part of mainland Australia; dark shaded areas indicate low-lying depressions within Bass basin that were filled with water. B) Rising sea levels submerged the western sill forming an estuarine environment 17500 years ago. C) Last connection 14000 years ago between Tasmania and mainland Australia via eastern landbridge, the Bassian Isthmus. D) Present day coastline with coloured regions indicating marine biogeographical provinces after Bennett & Pope (1953). Arrows indicate the flow patterns of major boundary currents in the area - the Zeehan Current (ZC, dashed) and the Eastern Australian Current (EAC, continuous) (Cresswell 2000, Ridgeway 2007). During the last glacial the ZC ceased its flow along the western Tasmanian coast while the EAC reached the southeastern tip of Tasmania (Nuernberg et al. 2004). Present day oceanographic conditions see both currents flow into Tasmania, although they are strongest at different times during the year.

The intertidal brown seaweed, *Hormosira banksii* (Turner) Descaisne (Fucales, Phaeophyceae), is abundant on rocky shores throughout southern Australasia, forming dense canopies and providing habitat for diverse communities (Coleman et al. 2011a, Schiel and Lilley 2011). In Australia, its distribution ranges from Albany (Western Australia) around the southern coast to Angourie (New South Wales) and the entire coast of Tasmania (Womersley 1967, Millar and Kraft 1994). *Hormosira* is a perennial and dioecious alga and fronds are fertile year round with gametes released on incoming tides (Osborn 1948, Levring 1949, Gunthorpe et al. 1995, McKenzie and Bellgrove 2008). Eggs are negatively buoyant and sink immediately after release and once fertilised by the motile sperm, the zygotes adhere quickly to the substratum (Forbes and Hallam 1979, Dunmore 2006). *Hormosira* recruits usually occur in close proximity to their parental stands (Dunmore 2006) as the direct-developing life history with no larval stage and the fast adhesion time result in limited capacity for dispersal (McKenzie and Bellgrove 2006, 2008). Although detached fronds can float, the contribution of rafted fronds to gene flow is believed to be low (McKenzie and Bellgrove 2008).

This study aimed to examine patterns of genetic structure and diversity of *Hormosira* in southeastern Australia using the Cytochrome Oxidase 1 (CO1) region of the mitochondrial genome as this gene provides a single stable marker of the maternal line. Even though CO1 is a conservative marker it has often been applied to investigate the relative influence of historic and contemporary processes in phylogeographic studies (e.g. Buchanan and Zuccarello 2012) or to explore contemporary habitat discontinuities (e.g. Fraser et al. 2010a). Moreover, mtDNA markers were successfully used to determine the origins of beach-cast kelp (Collins et al. 2010), as well as estimating the ranges of long-distance rafting in kelp (Nikula et al. 2010, Fraser et al. 2011). Given its limited capacity for dispersal, *Hormosira*

would be expected to show clear genetic structuring and limited mixing between populations. Previous studies on genetic structuring in marine species in southeastern Australia have focused mainly on the differentiation of eastern and western populations while the potential role of Bass Strait as a region of overlap for three biogeographic provinces has been largely ignored. Specifically, we explored the likely roles of historic and contemporary factors in determining divergence patterns in this intertidal seaweed in southeastern Australia. We further aimed to examine potential drivers of postglacial dispersal of *Hormosira* into Bass Strait.

Material and Methods

Sampling sites and collection

Mature fronds of *Hormosira banksii* were collected by hand from the intertidal at 19 sites across the seaweed's distributional range in southern Australia (see Table 5.1). Samples from the Tasmanian sites were collected between January and March 2013 except for samples from the Furneaux Islands (September 2014) and Port Davey (April 2015). All mainland Australian samples were collected in October 2015. Individuals were sampled along a 10 m transect with a minimum distance of 0.5 m between collected specimen. Samples were either frozen within 24h or stored in RNAlater for no more than three weeks prior to freezing. Between 11 and 30 individuals were sequenced per site.

DNA extraction and CO1 gene amplification

Frozen tissue samples were thawed and excess RNAlater removed by gentle shaking. A small piece of thallus tissue (<1 mm^2) was removed with a sterile scalpel, placed into a 2.0 ml centrifuge tube and homogenised in 600 µl of extraction buffer (4M urea, 1 % sodium dodecyl sulphate (SDS), 0.2 M sodium chloride and 1 mM

sodium citrate) using a micro-pestle driven by a battery drill (Speed: 0-750/min; Ozito Industries, Bangholme, Australia) (A. Bridle, unpublished data). Homogenates were placed on ice for 5 minutes, and then 300 µl of 7.5 M ammonium acetate was added, mixed by inversion, and centrifuged at 14, 000 rpm for 5 minutes at 4˚C. The supernatant was transferred into a new tube and DNA precipitated by addition of an equal volume of 100% isopropanol, mixed by inversion, and centrifuged at 14, 000 rpm for 8 minutes at room temperature. The resulting total nucleic acid pellet was washed in 500 µl 70 % ethanol, resuspended in 200 µl of molecular grade water (Sigma Aldrich, Castle Hill, Australia) and incubated at 55˚C for 10 minutes. Total DNA pellets were then further purified using a modified protocol for the DNeasy Blood and Tissues kit (Qiagen, Hilden, Germany). Briefly, 200 µl of DNeasy lysis buffer AL was added and the sample incubated for 10 minutes at 55˚C. A volume of20 µl of 100% ethanol was added and the total volume transferred to a DNEasy kit DNA binding silica column and centrifuged at 10,000 rpm for 30 seconds. Two washing steps were carried out with 500 µl of ethanol based buffers AW1 (1 minute at 10, 000 rpm) and AW2 (3 minutes at 10, 000 rpm), and the sample eluted in 50 µl elution buffer (10 mM Tris-HCL, 0.5mM EDTA).

An approximately 580 bp section of the mitochondrial CO1 gene was amplified from individual samples using primers *cox*1-789F and *cox*1-1378R (Silberfeld et al. 2010). PCR amplification was carried out in 50 µl volumes containing 1X NH_4 reaction buffer (Bioline, Alexandria, Australia), 0.2 mM dNTP's, 0.4 µM of each primer, 3 mM $MgCl_2$, 0.06 % bovine serum albumin (BSA) and 1.25 U BIOTaq polymerase (Bioline, Alexandria, Australia). PCR cycling consisted of initial denaturation for 4 minutes at 95˚C, followed by 35 cycles of: 30 seconds at 94˚C, 30 seconds at 50˚C and 60 seconds at 72˚C; and a final extension of 4 minutes at 72˚C. Successful PCR products were purified using UltraClean PCR Clean-up columns (Mo-Bio, Carlsbad, USA) and

sequenced using ABI Big Dye dye-terminator chemistry and the amplification primer (*cox*1-789F) by the Ramaciotti Centre for Functional Genomics at the University of New South Wales (Sydney, Australia).

Data analysis

Sequence electropherograms were manually corrected and compared to each other using the software Geneious (version 5.0.3, Kearse et al., 2012). Sequence variation and distinct mt-CO1 haplotypes were determined by iterative comparative alignment of all sequences obtained during the study. The program DNAsp (Librado and Rozas 2009) was used to calculate nucleotide diversity (π) and haplotype diversity (Hd) at each site and for the entire data set. Haplotype genealogies were constructed with the TCS method (Templeton et al. 1992) using statistical parsimony to describe population level structure (Clement et al. 2000) in POPART (http://popart.otago.ac.nz). Genetic divergence values were obtained using MEGA (MEGA version 7.0.14, Kumar et al., 2015). We applied Analysis of Molecular Variance (AMOVA, Excoffier et al., 1992) to test patterns of genetic structure within and among sites and calculated pairwise F_{ST} estimates were then calculated between sites to explore differences in population structures in Arlequin (Arlequin version 3.5.2.2, Excoffier & Lischer, 2010). Significance tests were derived from 1,000 random permutations and obtained p-values followed the Bonferroni correction for multiple comparisons. Non-metric multidimensional scaling (nMDS) (Clarke 1993) was used to visualise similarities between sites according to their haplotype composition. Haplotypes present at each site were used to examine whether distribution patterns in *Hormosira* resemble known biogeographic structures in southern Australia. Data was square-root transformed before Euclidean distances were calculated. Ordination analysis was performed in Primer v6 (PRIMER-E, Plymouth, UK).

Results

We obtained a total of 470 CO1 sequences for *Hormosira* sampled from 19 sites around southeastern Australia (Table 5.1). Eleven variable positions (all transitions) were found over the sequenced (and trimmed) 557 bp CO1 fragment (1.98% of sites) resulting in 12 different mtDNA haplotypes (Genbank accession numbers: KY020027-KY020038). Sequence divergence was low, ranging from one to four nucleotide substitutions (0.18 to 0.718 % sequence divergence); all haplotypes were distinguished from others by a single nucleotide substitution. Eight haplotypes were confined to single sample sites and four were singletons. Despite overall low nucleotide diversity (π= 0.574) haplotypes clustered into three major geographical groups, corresponding to 'eastern' (C-IV – C-VI), 'western' (C-II and C-III) and 'central' (C-I, C-VII – C-XII) areas within southeastern Australia (Figure 5.2, Figure 5.3). The central haplotype C-XII likely represents the shared ancestral haplotype as all of the other types radiated from the C-XII by one or two mutational steps (Figure 5.3).

Table 5.1: Sites where *Hormosira* was collected, and site abbreviations; the number of samples sequenced, haplotype/s found and genetic diversity (π: nucleotide diversity; Hd: Haplotype diversity) at each site.

Site	Abbr.	sequenced samples	Haplotype/s	genetic diversity	
				Hd	π
Port Campbell	PC	29	C-II, C-XII	0.379	0.00068
Marrawah	MW	24	C-II, C-XII	0.159	0.00029
Temma	TE	29	C-II, C-III	0.069	0.00012
Trial Harbour	TH	24	C-XII	0	0
Port Davey	PD	18	C-XII	0	0
Southport	SP	30	C-XII	0	0
Alonnah	AL	21	C-XII	0	0
Eaglehawk Neck	EN	24	C-XII	0	0
Shelly Beach	SB	23	C-XII	0	0
Four Mile Creek	FM	25	C-XII	0	0
Bay of Fires	BF	27	C-IV, C-XII	0.462	0.00083
Furneaux Group	FL	25	C-XII	0	0
Petal Point	PP	22	C-IX, C-XII	0.091	0.00016
Low Head	LH	11	C-XII	0	0
Rocky Cape	RC	24	C-I, C-XII	0.087	0.00016
Mornington Peninsula	MP	29	C-II, C-VII, C-VIII, C-IX, C-X, C-XII	0.751	0.00186
Cape Conran	CC	30	C-XI, C-XII	0.069	0.00012
Coledale	CD	28	C-IV	0	0
Nambucca Heads	NH	27	C-IV, C-V, C-VI	0.211	0.00039
TOTAL sequenced		470		0.574	0.00123

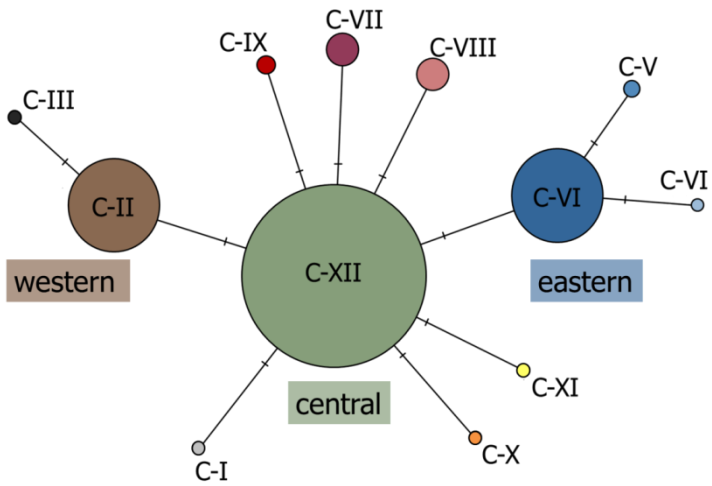

Figure 5.2: Haplotype network of 12 haplotypes found for *Hormosira* in southern Australia. Circle size is proportional to haplotype frequency and hatch marks indicate mutational steps between haplotypes.

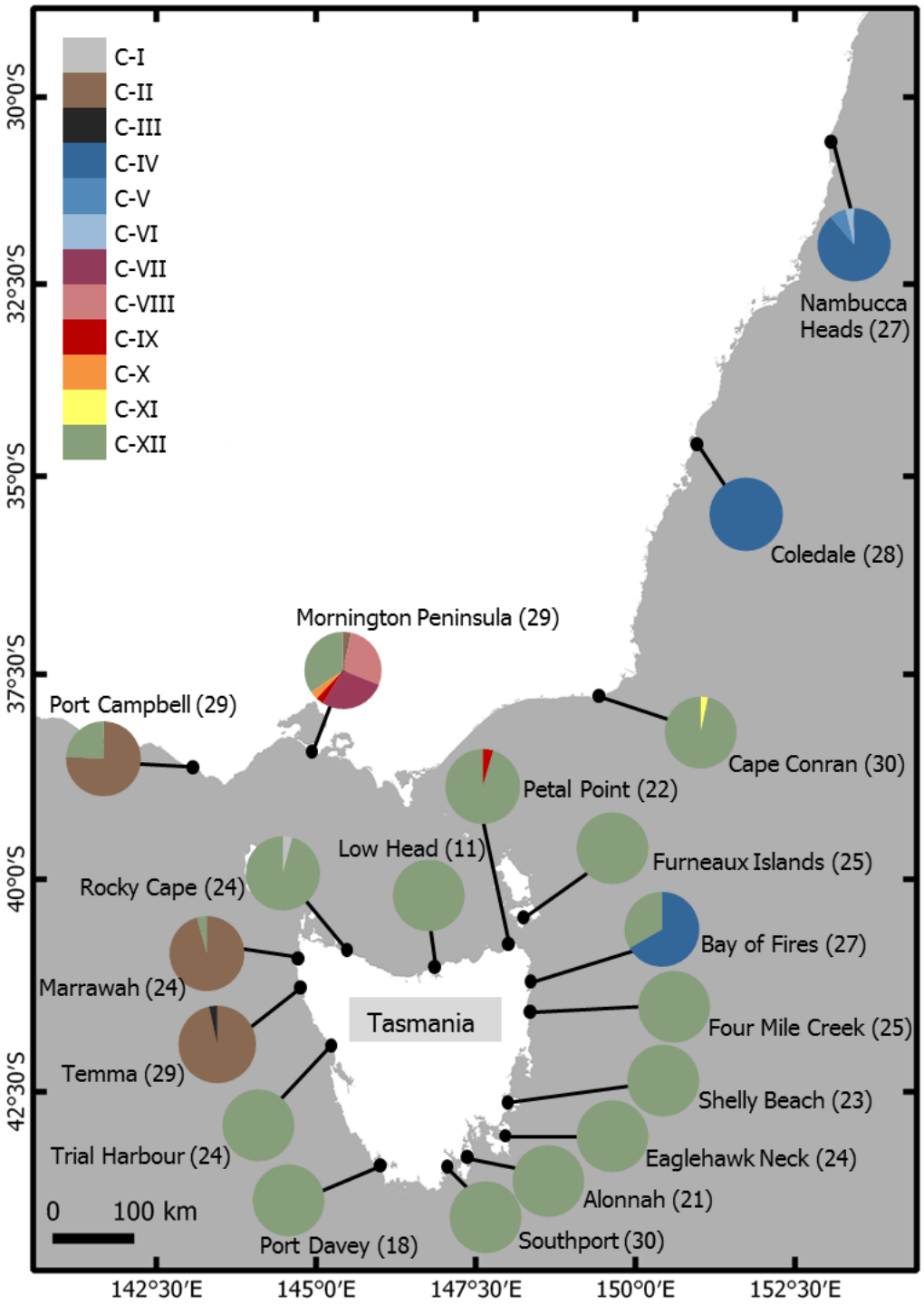

Figure 5.3: Distribution and frequency of *Hormosira* CO1 haplotypes in southeastern Australia. Pie charts on the map indicate relative proportions of CO1 haplotypes at each site.

The three most widespread haplotypes were C-XII (central; 65.75 %), C-II (west; 15.54) and C-IV (east; 11.06%) which together accounted for 92.35% of all haplotypes. No haplotype of the western group (C-II and C-III) occurred at eastern geographical sites, and all eastern-group haplotypes (C-IV – C-VI) were only found at eastern Australian coastal sites (Figure 5.3). Individuals of eastern and western haplotype groups differed by a maximum of four substitutions (0.36 – 0.72% divergence). Both, the most abundant haplotypes of the eastern (C-IV) and western group (C-II) were found at mainland and Tasmanian sites. The eastern and western haplotype distributions did not overlap across the study area indicating a clear east-west split (Figure 5.3). No haplotype occurred at all sites (Table 5.1, Figure 5.3) but the most common central haplotype C-XII was widespread. All other central haplotypes were restricted to sites within Bass Strait, between northern Tasmania and the southern stretch of the mainland Australia coastline (Figure 5.3). Notably, eight haplotypes were detected in the Bass Strait region but only one western haplotype individual and no eastern group haplotypes were detected (Figure 5.3).

Haplotype diversity was low at most sites with no diversity detected at 9 of 19 sites sampled. Mornington Peninsula (within Bass Strait) exhibited high diversity (6 haplotypes; Hd: 0.751), as did Nambucca Heads (3 haplotypes; Hd: 0.211) near the northern distribution limit of *Hormosira* (Table 5.1). No more than 2 haplotypes were detected at other sites, with Port Campbell western Victoria (2 haplotypes; Hd: 0.379) showing the highest diversity. Only four of 14 Tasmanian sites exhibited any haplotype diversity with the eastern coast site Bay of Fires (2 haplotypes, Hd: 0.462) the most diverse (Table 5.1).

AMOVA indicated that the majority (73.5%) of genetic variation was between populations (e.g. sites) while only 26.5% of variation was within populations (Table

5.2). Pairs of sites that were similar corresponded with the grouping in western, central and eastern geographic locations (Table 5.3). Pairwise F_{ST} indicated no difference in population genetic structure between the east coast sites Nambucca Heads and Coledale on the Australian mainland and Bay of Fires at the Tasmanian east coast. Similarly, Port Campbell located on the central mainland and Marrawah and Temma on the Tasmanian west coast were genetically similar. The remaining Tasmanian sites (and Cape Conran) showed no differences in genetic structure among their populations. However, CO1 haplotype frequencies at Tasmanian north coast sites (Petal Point, Low Head and Rocky Cape) were similar to Mornington Peninsula, although this was due to the overly conservative Bonferroni correction.

Table 5.2: Analysis of molecular variance (AMOVA) among and within sampled sites.

Source of variation	df	SS	Variance components	Percentage variation	P
Among sites	18	130.18	0.27570	73.53	<0.001
within sites	475	47.15	0.09926	26.47	
Total	493	177.33	0.037496		

P values are based on 1000 permutations.

Table 5.3: Pairwise F_{ST} estimates among sites where *Hormosira* was sampled. Bold values in shaded cells indicate significance after 1000 permutations and Bonferroni correction (cut off $p < 0.0002$) for multiple comparisons. Site abbreviations are as in Table 5.1; brown coloured sites indicate western region, green coloured sites refer to central region, and blue coloured sites show eastern region.

	MW	TE	TH	PD	SP	AL	EN	SB	FM	BF	FL	PP	LH	RC	PC	MP	CC	CD	NH
MW	-																		
TE	-0.13	-																	
TH	**0.87**	**0.90**	-																
PD	**0.82**	**0.87**	0.01	-															
SP	**0.89**	**0.91**	0.00	0.03	-														
AL	**0.87**	**0.90**	0.00	0.01	0.00	-													
EN	**0.87**	**0.90**	0.00	0.01	0.00	0.00	-												
SB	**0.87**	**0.90**	0.00	0.01	0.00	0.00	0.00	-											
FM	**0.76**	**0.83**	0.12	0.08	0.14	0.11	0.12	0.12	-										
BF	**0.70**	**0.79**	**0.52**	**0.43**	**0.55**	**0.51**	**0.52**	**0.52**	**0.43**	-									
FL	**0.88**	**0.90**	0.00	0.02	0.00	0.00	0.00	0.00	0.13	**0.53**	-								
PP	**0.83**	**0.88**	0.00	0.00	0.02	0.00	0.00	0.00	0.09	**0.49**	0.01	-							
LH	**0.83**	**0.88**	0.00	-0.03	0.00	0.00	0.00	0.00	0.06	**0.44**	0.00	-0.04	-						
RC	**0.83**	**0.88**	0.00	0.00	0.01	0.00	0.00	0.00	0.03	**0.49**	0.00	0.00	-0.04	-					
PC	0.04	0.14	**0.73**	**0.68**	**0.75**	**0.72**	**0.73**	**0.73**	**0.64**	**0.64**	**0.74**	**0.69**	**0.67**	**0.69**	-				
MP	**0.57**	**0.69**	**0.19**	**0.16**	**0.21**	**0.18**	**0.19**	**0.19**	**0.19**	**0.40**	**0.20**	0.17	0.12	0.18	**0.48**	-			
CC	**0.85**	**0.88**	-0.01	0.00	0.00	-0.11	-0.01	-0.01	0.11	**0.53**	-0.01	0.00	-0.04	0.00	**0.72**	**0.20**	-		
CD	**0.94**	**0.95**	**1.00**	**0.95**	**1.00**	**1.00**	**1.00**	**1.00**	**0.88**	0.26	**1.00**	**0.96**	**1.00**	**0.96**	**0.89**	**0.68**	**0.97**	-	
NH	**0.89**	**0.92**	**0.90**	**0.85**	**0.91**	**0.90**	**0.90**	**0.90**	**0.81**	0.23	**0.90**	**0.87**	**0.87**	**0.87**	**0.84**	**0.65**	**0.88**	0.02	-

The central haplotype C-XII, was absent from the eastern mainland coastline, despite its wide distribution and dominance along the Tasmanian coastline. This haplotype was the sole type occurring from central south-western to central southeastern Tasmania, over a distance of approximately 500km; however, neither the Tasmanian east nor west coasts were genetically homogeneous. The west coast north of Trial Harbour was dominated by western haplotype C-II and by central haplotype C-XII to the south; on the east coast a single site, Bay of Fires, was dominated by eastern haplotype C-IV (Figure 5.3).

The nMDS plot clustered sites into geographic groupings (Figure 5.4). Most Tasmanian sites and Cape Conran were very similar in the MDS reflecting the shared haplotype frequencies of those sites. Marrawah and Temma clustered closer to the Port Campbell population and formed an individual group while Bay of Fires was located closest to the eastern group (Figure 5.4). The Mornington Peninsula site was the most distant from all other sites due to its high number of unique haplotypes (Figure 5.4).

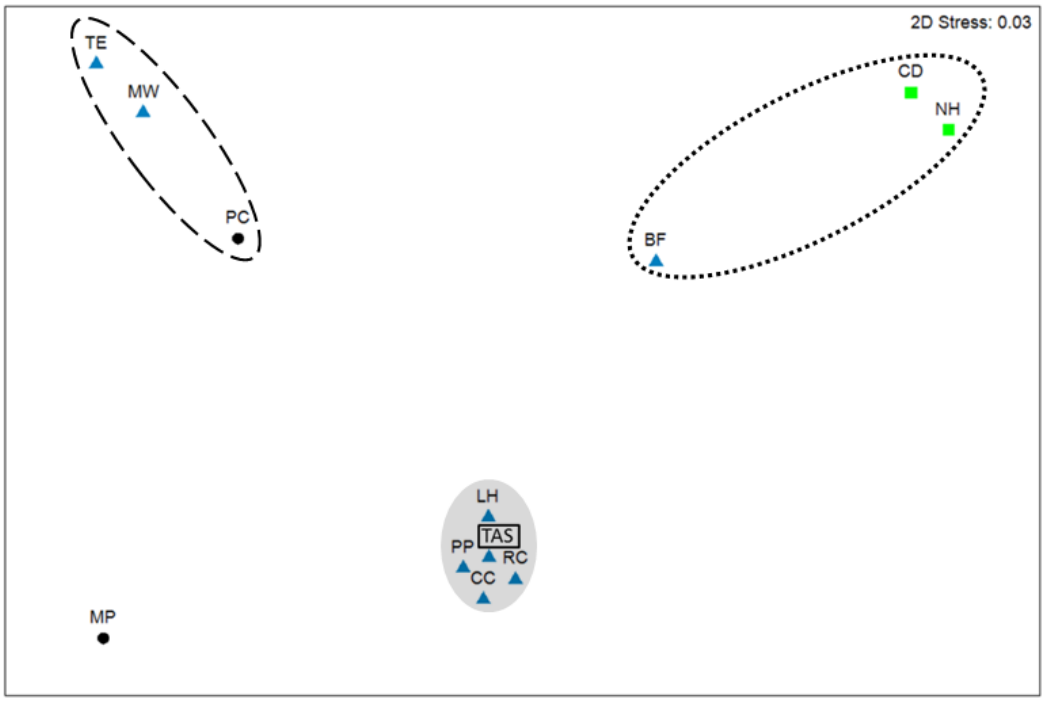

Figure 5.4: nMDS showing similarities between sites based on their haplotype composition. Site abbreviations as in Table 5.1; site symbols refer to the biogeographic province they are located in (circle = Flindersia, triangle= Maugea, square = Peronia). Squared box with TAS inscription represents Tasmanian sites (TAS) FM, SB, EN, AL, SP, FI and TH that are identical due to 100% dominance of haplotype C-XII. Dashed shape indicates western grouped sites, dotted shape encircles eastern grouped sites and grey shaded area shows central grouped sites.

Discussion

Historic conditions pre LGM

Time-calibrated brown algal phylogenies estimate that *Hormosira* diverged from sister taxa between 40-50 Ma years ago (Silberfeld et al. 2010, Kawai et al. 2015). The absence of closely related sister taxa and a temperate distribution restricted to Australasia (Womersley 1967) suggest that *Hormosira* may have evolved in the shallow seaway that was established between Australia and New Zealand around 60 Ma years ago, but before Australia separated from Antarctica (circa 30 Ma years

ago) (McGowran et al. 2000). The Southern Ocean and Antarctic region climate at this time was warmer and wetter than present day (McGowran et al. 2000). Australia gradually moved north, and Antarctica moved south, until the two landmasses completely separated approximately 20 Ma years ago. Opening of this seaway led to formation of the circum-polar current and a rapid cooling of the Antarctic climate (McGowran et al. 2000), presumably resulting in the extinction of *Hormosira* across the Antarctic and islands of the southern ocean, leading to its present day Australasian distribution.

The 'star'-like radiating-phylogenetic structure observed in our network analysis with haplotypes radiating from a relatively common central haplotype by only one or two mutational steps is a strong indicator of range expansion in populations (Avise 2000). However, if *Hormosira* had been established along the southern Australian coastline for approximately 20 Ma years, Australian ancestral populations would be expected to exhibit greater levels of genetic diversity than observed in our study, indicating that substantial population bottlenecks have occurred in recent palaeohistory. Average CO1 divergence rates of brown algal taxa comprise approximately 3.3 substitutions per million years (Fraser et al., 2010b; Silberfeld et al., 2010). Assuming a similar rate for *Hormosira* suggests the three CO1 haplotype groups diverged between 380 000 and 180 000 years ago. Consequently, it is likely that all three haplotype groups (eastern, western and central) were present in southeastern Australia before the peak of the last Pleistocene glaciation.

The Australasian climate during the Pleistocene (2.6 Ma years – 10 000 years ago) was characterised by warm interglacial and cold glacial periods, but climatic shifts were more severe at higher latitudes (Byrne 2008). The higher haplotype diversity of the central group is indicative of a widespread previously abundant and diverse

ancestral population prior to the emergence of either western or eastern haplotype groups. Harsh climatic conditions during glacial periods may have led to the contraction of *Hormosira* to warmer refugia (e.g. Byrne, 2008). Subsequent re-colonisation and range expansion during favourable inter-glacial periods may then have originated from reduced refugial populations leading to a series of population bottlenecks and a loss of haplotype diversity (Hewitt 1996). Alternatively, increased selection pressures, such as sharp temperature gradients in southeastern Australia, could have led to adaptation, promoting the formation of a colder-water *Hormosira* group coincident with the Maugean province, and two warmer water groups coincident with the Flindersian to the west and Peronian to the east (e.g. Li et al., 2013).

During the last 100 000 years Tasmania has been mostly isolated from the Australian mainland due to the inundation of the shallow water basin that is present day Bass Strait (Lambeck and Chappell 2001). Nevertheless, a land connection periodically existed on its eastern side for several short intervals during this time, providing a re-occurring barrier to movement and colonisation of marine species before very low sea levels re-established a land-bridge between Tasmania and mainland Australia during the LGM (Lambeck and Chappell 2001). Consequently, marine species were subjected to relatively short periods (one to several thousands of years) when the emerged landmasses of the eastern Bass strait sill separated eastern and western coasts of southern Australia (Lambeck and Chappell 2001) resulting in separation and genetic divergence of eastern and western populations before the last glacial maximum (Waters 2008).

Conditions during last glacial

At the peak of the last glacial, the Zeehan current (ZC) ceased its southerly flow down the western coast of Tasmania due to the northward retraction of the Subtropical Convergence Zone (STC) (to approximately 42° S) which brought cold Antarctic waters to the Tasmanian west coast (Nürnberg and Brughmans 2004). Retraction of the ZC further resulted in an eastwards rain-shadow effect combined with strong westerly winds that led to glacial arid conditions on the Tasmanian east coast (Macphail 1979). Sea temperatures also decreased along the Tasmanian east coast, although the temperature drop was less severe than the cooling of the adjacent land (Barrows and Juggins 2005). Intertidal organisms like *Hormosira* would have been affected by both decreasing ocean temperatures and glacial terrestrial conditions which may have caused extensive loss of *Hormosira* along the central and southern parts of Tasmania's east and west coasts. Western haplotypes of *Hormosira* only extend as far south as Temma on the northern part of the Tasmanian west coast; south of this point only central haplotypes were detected. This break point coincides with the expansion of the STC to approximately 42° S during the LGM (see Nürnberg & Brughmans, 2004) and implies that *Hormosira* persisted north of the STC during the LGM but not to the south of the STC due to the much colder water temperatures.

When sea levels were low during the LGM, deep depressions within the Bass basin filled with water and formed a large brackish lake with varying salinities (Blom and Alsop 1988). This lake was isolated within the central basin although a broad river valley with some smaller lake structures provided a connection to the Southern Ocean (Lambeck and Chappell 2001). Based on connectivity patterns between meadows of the seagrass *Posidonia australis*, a marine glacial refuge is thought to have existed in modern day Bass Strait region during the LGM (see Sinclair et al.,

2016). Given the high diversity of central group haplotypes at Mornington Peninsula, including three haplotypes not found at any other site, a glacial refuge may have existed in the vicinity of the valley system from which post-glacial re-colonisation was initiated (e.g. Provan et al., 2005). Interestingly, similar diversity patterns of the red seaweed *Palmaria palmata* (Provan et al., 2005) and the brown seaweeds *Ascophyllum nodosum* (Stam et al. 2001) and *Fucus serratus* (Coyer et al. 2003) provide similar evidence of a glacial refuge in the English Channel during the LGM. Although the English Chanel was dry land during the LGM, low lying depressions and trenches, similar to those within the Bass basin, are proposed to have persisted as marine lakes (Provan and Bennett 2008). Nevertheless, survival and persistence in such refugia is dependent on suitable habitat and high genotypic plasticity to tolerate the physical environment. Fucoid species in the Baltic Sea successfully reproduce at salinities of ≥ 7 PSU (Malm et al. 2001) and *Hormosira* is commonly found in estuaries and rockpools. Thus fucoid species appear capable of tolerating both high thermal and salinity stress (Kain, 2015) expected to occur in marine lake refugia.

While water conditions within the Bass basin are not known, *Hormosira* populations may have persisted in the area until after the LGM when sea levels rose to form an estuarine environment around 17 500 years ago (Lambeck and Chappell 2001). Initially water entered the estuary from the west via the broadening valley system near the present location of Mornington Peninsula but this mouth gradually opened with the continuing rise in sea levels (Lambeck and Chappell 2001). As a result, more habitat became available and may have paved the way for a rapid range expansion of the dominant central group haplotype in the widening Bass estuary, still largely isolated by exposed landmasses to the east and west. Previous studies have assumed a glacial refuge existed for marine organisms further to the west near

the modern day Great Australian Bight (Waters and Roy 2003) and concluded postglacial re-colonisation occurred from the west (Waters 2008). If the direction of re-colonisation into Bass Strait (and subsequently into Tasmania) was from the west and driven by a re-intensifying ZC down the Tasmanian west coast, one would expect western haplotypes to be more broadly distributed or dominant within Bass Strait, as on the northern section of the Tasmanian west coast. However, eastern and western haplotypes are absent from modern-day Bass Strait indicating that re-colonisation of the Tasmanian coast was instead initiated from within/near the Bass basin.

The continuous rise in sea levels reduced the former landbridge to a small isthmus (the "Bassian Isthmus") on its eastern side until approximately 14, 000 ago (Figure 1; Lambeck & Chappell, 2001). As a result, the central haplotype group could progressively re-colonise the newly emerged habitat and further expand its range along the western shores of the Furneaux Island group (eastern Bass Strait) and eventually along the east coast as the Bass Strait seaway opened fully. While most sites (except Mornington Peninsula) within Bass Strait are dominated by haplotype C-XII, the underlying reasons for the continued dominance of this haplotype remain unclear. Theoretically, favourable conditions after the LGM may have facilitated rapid expansion enabling the first migrants to successfully establish colonies before other types arrive while competitive exclusion leads to a loss in diversity as migration continues (Hewitt 1996). Nonetheless, the rare haplotype C-IX was found at both Mornington Peninsula and Petal Point, a site on the Tasmanian north coast relatively close to the last connection of the ancient Bassian isthmus. This may reflect a remnant of the central haplotype group diversity within the Bass basin, supporting a proposed expansion route along the western shore of the narrowing landbridge on the eastern side of Bass Strait that is now the Furneaux Island chain.

Re-colonisation of the remainder of the Tasmanian coastline would then have proceeded from populations established on the northern coast. The abrupt break-point between Temma and Trial Harbour at the west coast, and the existence of monomorphic C-XII haplotype populations *Hormosira* around the entire southern half of Tasmania indicates that dispersal along the west coast occurred in a northerly direction opposite to prevailing flow of the ZC. *Hormosira* lacks a motile life-cycle stage which restricts gamete dispersal to a scale of metres. Given this low dispersal capacity, northward dispersal may be facilitated by a combination of local near-shore counter-currents and wind patterns (Hawes 2008) indicating that longer distance dispersal of *Hormosira* proceeds by a stepping stone model, with recruitment restricted to nearby locations within each generation (Kimura and Weiss 1964). Together with the observed haplotype distribution, re-colonisation of the central and southern Tasmanian coastline appears to have proceeded in a predominantly clockwise direction along the eastern Tasmanian coast.

While *Durvillaea potatorum* has similar life-history traits to *Hormosira* (Clayton 1990) mtDNA diversity and distribution patterns in southern Australia differ between the two species (e.g. Fraser et al., 2009). *D. potatorum* inhabits exposed and wave-swept coasts with steep intertidal gradients in the cool temperate waters of Australia but is absent from the Tasmanian north coast (Edgar 1997). The family Durvillaeaceae are characterised by a bulky structure with massive fronds and long and wide stipes (Edgar 1997). Buoyant fronds of the closely-related southern bull kelp, *Durvillaea antarctica*, can disperse distances of several hundred kilometres via ocean currents (Collins et al. 2010) facilitating long distance dispersal and gene flow (Fraser et al. 2010a). While rafting fronds of *Hormosira* can also drift long distances, this strategy is thought to be less successful as beach casted material predominantly lands on sandy (and thus unsuitable) bottom (McKenzie and Bellgrove 2008).

Reproductive success also requires that both male and female gametes are in immediate vicinity. In contrast to *Hormosira*, *Durvillaea* species often develop conjoined holdfasts of neighbouring individuals (Fraser et al. 2011) which may enhance reproductive efficiency through co-dispersal of male and female rafted thalli (Collins et al. 2010).

Contemporary barriers

The mt-CO1 haplotype discontinuities observed in our study indicate strong and continuing barriers to *Hormosira* gene-flow into Bass Strait from both westerly and easterly directions. Western haplotypes are virtually absent from Mornington Peninsula and absent at all other Bass Strait sites. Two co-dominant haplotypes C-VII and C-VIII at Mornington Peninsula were not detected at Cape Conran to the northeast of Bass Strait – a site dominated by haplotype C-XII. Those sites are separated by the extensive sandy habitat of Ninety Mile Beach, an area shown to impede dispersal of other rocky intertidal species (Ayre et al. 2009). The complete absence of western group haplotypes along the southwestern coastline of Tasmania indicated that there is also a significant and long-term dispersal barrier for *Hormosira* between Temma and Trial Harbour. The northern part of the Tasmanian west coast is an area with some of the highest energy beach systems in Australia (Short 2006b). The Sandy Cape dune system extends for approximately 40km of the coastline north of Trial Harbour creating a high energy and unstable environment potentially capable of inhibiting successful settlement of *Hormosira* zygotes and other rocky reef species. Moreover, geography of the north-south shoreline changes direction south of Sandy Cape and turns southeast creating different flow and wind dynamics along the shore (Short 2006b) that may further reduce the likelihood of zygote dispersal across the Sandy Cape area.

The dominance of the eastern group haplotype C-IV at Bay of Fires on the Tasmania eastern coast may represent a relic of more widely distributed eastern haplotypes that existed along the continuous eastern coastline prior to the most recent glacial maximum. With rising sea levels, the land bridge was submerged isolating the north-eastern Tasmanian sites from eastern Victoria. Subsequent stepping-stone re-colonisation of the majority of the eastern Tasmanian coastline by central haplotype C-XII may have replaced eastern haplotypes, although it remains unclear why the eastern haplotype is confined to only the northern part of the east coast. Prominent topographic features between the Bay of Fires and Four Mile Creek include St Helens Point which backs the flood tide delta of Georges Bay followed by an extensive dune system (approximately 35 km) all the way south to Four Mile Creek (Short 2006b). Constant river run-off through the estuary might act as a barrier to gene flow and alter local coastal oceanography (Watts and Johnson 2004) while the long sandy beaches may further handicap gene flow. Both genetic breakpoints for Tasmanian *Hormosira* are adjacent to long sandy beaches, however it remains unclear whether the effect of unsuitable habitat is reinforced by other oceanographic features.

Linkage to biogeographic provinces

Despite the low genetic diversity, the distribution of haplotypes correlate with the ranges of biogeographic provinces described for southern Australia. The central haplotype (C-XII) distribution corresponds well with the Maugean province while the eastern and western haplotype distributions compare well with the other two provinces Peronia (east) and Flindersia (west) (see Figure 4). Bennett & Pope (1953) postulated that the Bass Strait represents a merging area for Peronia, Maugea and Flindersia. Convergence zones between provinces often match disjunctions in marine populations (Li et al. 2013) and this also appears to be the case for *Hormosira*. Approximately two thirds of the individuals sampled at

Mornington Peninsula belonged to haplotypes found nowhere else, and no western haplotypes were found at Tasmanian north coast sites. Given that Bass Strait is a shallow water basin with weak currents (Coleman et al. 2013), it is reasonable to assume that gene flow may be slow if not restricted to either side within Bass Strait (east, west, south), thus reinforcing biogeographic boundaries. However, biogeography is highly influenced by species abundance, community structure and habitat composition (Connell and Irving 2008). Consequently, biological and ecological factors help maintaining divergence patterns at a species-level (Teske et al. 2016) resulting in complex interactions of a species' ecology with phylogeography.

While *Hormosira* shows large morphological variation across its distributional range (Mueller et al. 2015) the phenotypic variation appears uncoupled from the genotypic variation we observed in the CO1 gene. *Hormosira* at the northern Tasmanian sites have a short, 'bushy' phenotype compared to the rest of Tasmania (see Mueller et al., 2015) but these sites share the dominant central CO1 haplotype. The decoupling of morphology and genetic patterns is consistent with the kelp *Macrocystis* which has similarly low CO1 gene diversity (divergence of 1.8% in *Macrocystis* vs. 1.98% in *Hormosira*) despite huge variation in morphology (Macaya & Zuccarello, 2010). Although the goal of this present study was to assess genetic variation in *Hormosira*, our findings indicate a role for environmental factors as drivers for phenotypic variation, that the CO1 gene is not variable enough at the population level to discern population level differences (e.g. Macaya & Zuccarello, 2010), or that CO1 variation is selectively neutral and unlikely to reflect the local adaptation in *Hormosira* (R. Mueller, unpublished data).

Overall, the genetic structure of *Hormosira* in southeastern Australia is organised into three major haplotype groups (western, central, eastern) which likely diverged during events and environmental changes prior to the LGM. Subsequent re-colonisation of Tasmania by *Hormosira* after the LGM was largely from within or near modern day Bass Strait driven by the central haplotype C-XII that may have persisted throughout the most recent Pleistocene glaciations in refugia within or near modern day Bass Strait. Current boundaries and break-points between haplotypes may be maintained by a combination of a low dispersal capacity in *Hormosira*, major habitat barriers due to stretches of unsuitable habitat, and complex oceanographic conditions at disjunct sites. While some of the patterns may be influenced by major boundary currents, the ZC and EAC do not appear to be the main mechanisms for gene flow between populations. Instead, our data suggest that local habitat barriers are important isolating mechanisms for direct developing species such as *Hormosira*, and operate over shorter spatial scales than previously thought (e. g. the 35-40 km of Sandy Cape and Peron dunes area). Generalising across large spatial scales is thus difficult without an understanding of the local microenvironment and habitat availability. The clear genetic discontinuities observed in our study emphasise that *Hormosira* populations are genetically restricted with low levels of gene flow between sites. However, analyses using more variable markers such as microsatellites could determine fine-scale population connectivity across those spatial scales and more precisely establish the contemporary gene flow between Tasmanian and mainland populations.

Chapter 6

General discussion

Algae are the dominant habitat-forming organisms on temperate reefs in Australia and contribute greatly to ecosystem functioning (Wernberg et al. 2012, Bennett and Wernberg 2014, Bennett et al. 2015). To guarantee this functioning in the future we need to know how habitat-forming organisms respond to environmental change and whether their responses are consistent across varying habitats. Within species, populations respond differently to changes in their environment and consequently, species capacity to persist in a changing environment will be influenced by the diversity of responses at the population level (Araújo et al. 2011). However, to understand the consequences of population structuring and to assess its impact on ecosystems we need to identify the spatial scales at which populations may differ (Coleman and Muhlin 2008).

In this thesis I detected substantial morphological variation in the intertidal seaweed *Hormosira banksii* from small (meters apart) through to large spatial scales (10s of km to 100s of km apart) (i.e. Chapter 2). In particular, *Hormosira* from the Tasmanian north coast showed a distinct morphology, characterised by smaller vesicles and shorter fronds compared to *Hormosira* from the other regions. I further tested for relationships between morphological and environmental variation and found that regional morphological patterns can be best predicted by differences in tidal regimes between those coasts. The results from Chapter 2 paved the way to test the influence of the environment and, in particular, the different tidal dynamics between regions in generating phenotypic differences in *Hormosira* populations. Using transplantation experiments, I explored the potential for environment-dependant phenotypes and discovered the capacity for *Hormosira* to show a plastic response to changes in the environment was strongly affected by the individual's site of origin and that responses were population-specific, trait-dependant and indicative of local adaptation (i.e. Chapter 3). I also determined that *Hormosira*

embryos of northern origin had greater survivorship and growth in their local north coast environment compared to the foreign east coast environment, further reinforcing the evidence for local adaptation in the north coast morph from early in its life (i.e. Chapter 4). Genetic variation in *Hormosira* in southeastern Australia was characterised in Chapter 5 and demonstrated phylogeographic groupings corresponding with the three existing biogeographical provinces in this region. Historic break points appeared retained and were reinforced by modern day dispersal barriers emphasising the existence of isolated populations with low levels of gene flow.

Relationship between morphology and environment

In chapter 2, I found broad morphological variation in *Hormosira* between different regions in Tasmania. The hierarchical sampling design between regions, sites within those regions and zones at each site provided quantitative evidence of morphological variation in *Hormosira* across these scales and demonstrated that regions were the largest contributor to morphological differences. In particular, north coast individuals shared a generally smaller and more branched morphology that appeared to be linked to differences in regional tidal patterns. In Chapter 3, I further explored the regional tidal dynamics in Tasmania and found that the semi-diurnal tides at the Tasmanian north coast result in more frequent and longer emersion time, and greater temperature fluctuations, for intertidal communities compared to the adjacent east coast highlighting broad environmental differences between these coasts. Because *Hormosira* populations on the north coast were exposed to the air more often, they are likely to suffer greater emersion stress compared to populations on the east coast. Numerous studies have identified emersion stress as a factor affecting the fitness and morphology in intertidal seaweeds yet most of these studies have focussed on comparisons between different

heights on the shore (e.g. Wright et al. 2004, Williams & Dethier 2005, Hays 2007) or analysed latitudinal variation across large spatial scales (e.g. Lago-Lestón et al. 2009, Zardi et al. 2013). Little previous work has examined the role of tidal dynamics at smaller scales. In this context, Tasmania represents a comparatively unique system where contrasting tidal regimes operate within a small latitudinal gradient and consequently habitats in different regions have relatively similar mean sea surface and air temperatures.

I then examined whether the increased levels of emersion at the north coast correlated with the smaller and somewhat 'bushy' *Hormosira* morphology (i.e. Chapter 3). This highly branched morphology at the north coast creates a compact and structurally complex canopy that likely protects *Hormosira* individuals within and beneath the canopy from various physical stressors such as extreme temperatures (R. Lewis, unpublished data), desiccation and radiation during emersion (Helmuth et al. 2002). Specifically I tested whether juveniles reciprocally transplanted between the north and east coasts adjusted to the novel environmental conditions to match the morphology of the individuals at the recipient site. Surprisingly, transplanted individuals demonstrated only weak evidence of plasticity and the observed responses appeared trait-dependant and population-specific. Performance gradients were observed between replicated populations from different sites of the same coast, highlighting the impact of environment and population on the expressed phenotype and suggesting that selection pressures act on populations independently.

Nevertheless, *Hormosira* juveniles from the Tasmanian east coast appeared to have greater capacity for plasticity than north coast juveniles indicating that eastern phenotypes have the potential to adjust to novel environmental conditions.

Moreover, Chapter 4 demonstrated that the survivorship of eastern origin embryos did not decrease when they were out-planted to the unfamiliar environmental conditions at the north coast. Thus, both of these transplant experiments revealed that eastern phenotypes were able to tolerate the novel conditions suggesting they possess more flexible phenotypes (Reusch 2014). Combined results from Chapter 3 and 4 indicate that eastern origin *Hormosira* may be a habitat generalist with a broad tolerance of a wide range of environmental conditions (Gilchrist 1995). However, the success of habitat generalists also depends on their resilience to environmental change (Hoffmann and Sgrò 2011) and thus the multivariate phenotype of east coast individuals could be negatively affected as their plastic response was trait-dependant. For example, fixed branching traits (i.e. Chapter 3) could possibly lead to future fitness trade-offs in the eastern origin juveniles on the north coast by stopping them gaining surface area even though size and vesicle traits may change. Due to the very slow growth of *Hormosira*, prolonged observation into later development stages was not feasible within the experimental time-frame of this thesis, however, longer-term studies would assist in estimating the longer-term relative fitness of eastern origin transplants at north coast habitats. In addition, measurements of photosynthetic activity *in situ* could be beneficial in estimating the performance of phenotypes in intertidal seaweeds and comparing emersion resilience between local and foreign ecotypes (e.g. Zardi et al. 2011).

Likewise, although the risk of desiccation is greater during summer, the warmer temperatures at the north coast likely promoted growth for all out-planted embryo populations (Chapter 4). It remains unclear whether a similar response of enhanced growth would have been observed during a season with less favourable temperatures or during periods with even higher temperatures (Helmuth 2002, Helmuth et al. 2006, 2011). Therefore equal growth across different regions

detected in *Hormosira* populations from the east coast should be interpreted carefully and further studies with seasonal replication are required to reach robust conclusions on the performance of eastern origin embryos transplanted outside their native region.

Local adaptation

The pronounced interactions between environment and population for growth of juveniles and survivorship in embryos (i.e. Chapter 3 & 4) are highly indicative of local adaptation where local populations developed traits that were advantageous in their local environment regardless of their performance in other environments (Kawecki and Ebert 2004). In particular, north coast juvenile *Hormosira* individuals scarcely changed their morphology after transplantation to the east coast (i.e. Chapter 3) indicating that either morphological traits are fixed in north coast populations or that environmental pressures at the east coast may have not been strong enough to elicit a plastic response. Furthermore, out-planted embryos of northern origin demonstrated a distinct home-habitat advantage with high survivorship and growth in their local habitat (i.e. Chapter 4). However, there were declines in both of these traits when northern embryos were out-planted to east coast sites indicating a trade-off in the foreign habitat (Hereford 2009). These results suggest that different levels of environmental stress have promoted divergent selection in *Hormosira* and provide good evidence for local adaptation in populations from the north coast (e.g. Van Tienderen 1991, Kawecki & Ebert 2004, Hays 2007, Walter et al. 2016). Longer and more frequent emersion at the north coast increases the risk of desiccation and imposes populations to more extreme fluctuations in temperature and light (Davison and Pearson 1996, Helmuth et al. 2002, Williams and Dethier 2005) and may ultimately lead to population differentiation (Hays 2007, Zardi et al. 2013).

Interestingly, when comparing relative growth rates (RGR vesicle) of northern juveniles transplanted to the east coast with local (eastern) individuals, the northern transplants perform equally or slightly better than eastern individuals. I found similar results for embryo survivorship and northern origin embryos had a slightly higher survival compared to local eastern embryos, but showed marginally lower growth rates than local eastern embryos. Thus, transplanted north coast individuals that are adapted to high emersion pressures did not experience a fitness trade–off relative to the populations in the foreign habitat where there is less emersion. However, no clear consensus exists on whether fitness trade-offs are associated with local adaptation (Fry 1996) and there are a number of experiments demonstrating that a reciprocal home advantage comes without fitness losses (reviewed in Hereford 2009). Nonetheless, the interpretations of local adaptation in embryos from the transplant experiment should be approached cautiously given the short duration of the experiment. High mortality is common in early life stages of fucoids (Wright et al. 2004, Dunmore 2006, Schiel and Foster 2006) and was also encountered in this study. Earlier measurement intervals (e.g. after eight or ten days instead of 30 days) may have provided more conclusive evidence of differences in embryo's performance. However, the logistics of long travel times to the different sites, combined with high variability in the occurrence of low tides that were long enough to take measurements and return tiles to their position before the incoming tide, limited the period over which data could be collected.

Transplants experiments provide a powerful tool to detect local adaptation although inherited plasticity in field-collected individuals may confound the interpretation of results (Agrawal et al. 1999, Sanford and Kelly 2011). To reduce the risk of these confounding effects out-planting offspring from individuals bred for several generations (ideally two or more) in the laboratory have been proposed (Kawecki

and Ebert 2004, Sanford and Kelly 2011). This has been applied in local adaptation experiments for terrestrial plants with short generation times (Hall and Willis 2006, Walter et al. 2016) but can be impractical when working with perennial organisms such as *Hormosira* which usually take many months (or even years) to produce their first generation offspring (e.g. Clarke & Womersley 1981). By culturing embryos under identical laboratory conditions for seven days I tried to eliminate the impact of site-specific environmental conditions on individual development, although maternal effects may have still been present (Hays 2007). Nevertheless, higher survivorship and growth of both northern *Hormosira* populations clearly indicates a home-habitat advantage due to adaptation and not by acclimatisation (plasticity).

Interestingly, in a previous breeding experiment with *Hormosira*, McKenzie and Bellgrove (2006) noted slower development of embryos from Tasmania compared to embryos from Victoria. However, their Tasmanian samples originated from the Tasmanian north coast and thus probably reflected the small 'bushy' phenotype. This study also described that Tasmanian samples had smaller receptacles, which are most likely a function of generally smaller vesicles. I did not detect any regional (or even local) differences in female gamete size from Tasmania (Chapter 4). Nonetheless, comparisons of natural recruitment and development between contrasting regions in Tasmania or across latitudinal gradients (Australian mainland vs. Tasmanian populations) could contribute further knowledge to emersion resilience in different populations. In particular, comparisons involving the Victorian coastline would be of high interest as this shore is on the opposite side of Bass Strait to northern Tasmania, providing a replicated system to test the influence of tidal regimes in this region. Moreover, the small *Hormosira* morph, typical for the north Tasmanian coast, also occurs on the northern side of Bass Strait at Mornington Peninsula in Victoria.

Overall, the transplant experiments confirmed the influence of regional processes on local populations but also highlighted some population-specific responses in *Hormosira* between sites. Small-scale differences in physical conditions between sites can create microenvironments that promote selective divergence (e.g. Roberson & Coyer 2004, Hays 2007) and thus knowledge of the local conditions is important and needs to be included to improve ecosystem management across a species' range. The work in Chapters 2, 3, and 4 revealed that population structure in *Hormosira* cannot easily be generalised from broad-scale regional patterns as different populations are likely to respond differently to changes in the environment. Thus, care must be taken when trying to predict performance and persistence in widespread habitat-forming species such as *Hormosira*.

Genetic variation and population structure

While local adaptation can lead to species differentiation I did not find any evidence for genetic variation between *Hormosira* ecotypes. The CO1 marker did not detect a link between genetic diversity (Chapter 5) and the multivariate phenotype (Chapter 2). Phenotypes from the north coast sites Rocky Cape, Low Head and Petal Point varied greatly within their group but were also distinct from the other regions (e.g. northeast, southeast and west) due to their 'bushy' morphology (i.e. Chapter 2). Yet, with the exception of two individuals, these northern sites all possessed the central group mitochondrial CO1 haplotype C-XII that overwhelmingly dominated all Tasmanian sites except Marrawah, Temma and Bay of Fires. However, based on CO1 sequence variation, I found a clear segregation into three major haplotype groups (western, central, eastern) in *Hormosira* across southeastern Australia. The level of mitochondrial variation between the three haplotype groups suggested they diverged more than 100 000 years ago and persisted through the extensive climatic changes of the Pleistocene epoch. Consequently, the structuring into western,

central and eastern haplotype groups likely existed well before the last glacial maximum. Historic climatic change often helps predict the future resilience of a species under environmental change and populations that were able to tolerate and persist under stressful climatic conditions in the past could be more resilient to changes in the future (Padilla-Gamino and Carpenter 2007, Harley et al. 2012).

Surprisingly, re-colonisation into Bass Strait after the LGM was not driven by expansion of populations (western haplotypes) from the west when the Strait re-opened to the west, but instead by the ancestral central haplotype (C-XII) perhaps from a glacial refuge near or within present day Bass Strait (Chapter 5). Further studies therefore should increase sampling effort in this region including the most likely route of stepping-stone re-colonisation of Tasmania - the Furneaux Island group and smaller rocky outcrops north to Wilson's Promontory on mainland Australia. While many of these sites are remote and not easily accessible they may provide a much clearer picture of the post-LGM re-colonisation pathway and the origins of a number of rare haplotypes unique to the northern Bass Strait area. Distinct break-points between dominant haplotypes were detected on the Tasmanian coast indicating that gene flow in *Hormosira* is low (e.g. Coleman et al. 2011a) and may be maintained by a combination of low dispersal capacity and major habitat barriers. Break points in haplotype distribution patterns suggest that long stretches of unsuitable habitat are successful barriers to dispersal. Restrictions to gene flow can affect the adaptability of populations to increasing environmental stressors and reduce ecological resilience in a changing environment (Hughes et al. 2008, Hoffmann and Sgrò 2011).

CO1 is a component of the electron transport chain in mitochondria and thus carries a key physiological function for the organism and is unlikely to be linked with

morphological variation as those markers are presumed to be selectively neutral (Reusch 2001). As a consequence, the use of more variable and rapidly evolving loci or markers would allow estimates of gene-flow among the different haplotype and populations. Relationships between genetic and morphological variation may perhaps be more easily determined by examining variation in genes connected with developmental processes or physiological performance in *Hormosira*.

While the mitochondrial CO1 variation in *Hormosira* provides an overview into the evolutionary patterns of *Hormosira* in southeastern Australia, a better estimate of population connectivity and gene flow at small spatial scales is required to predict the evolutionary response of individuals (Etterson and Shaw 2001). Highly polymorphic DNA markers such as microsatellites or Single Nucleotide Polymorphisms (SNPs) have the potential to detect genetic diversity that may correlate with variation in selectively important genes. Moreover, an increasing number of studies have demonstrated that distinct morphological variation between habitats with spatially varying selection regimes occurs despite no differences in neutral DNA markers (see Zardi et al. 2013). For example, traditional markers such as nuclear ITS and mitochondrial DNA were not able to distinguish between *Fucus vesiculosus* and *Fucus spiralis* (Serrão et al. 1999, Coyer et al. 2006b), two fucoids inhabiting the Atlantic coastline of southern Europe that only vary in their vertical distribution on the shore (Zardi et al. 2011), while microsatellites demonstrated clear genetic isolation between the two species (Billard et al. 2010). Interestingly, *F. spiralis* is found higher on the shore (and thus exposed to longer emersion times) and has a smaller morphology and higher emersion resilience compared to *F. vesiculosus* which is found lower on the same shores (Zardi et al. 2011). This obvious similarity in morphology patterns between *Hormosira* and the *Fucus* complex begs for future genetic analyses using highly variable markers. Gaining a better

understanding of genetic variation at the population scale will not only be important for understanding evolutionary potential with respect to changes in their environment (Hoffmann and Merilä 1999) but also can have important implications for strategies to conserve species' biodiversity.

Conclusion

The data presented in this thesis showed large differences in morphology of the habitat-forming intertidal seaweed *Hormosira* and importantly, distinct phenotypes followed a broad regional pattern (west ≠ north ≠ east) and were best correlated with the tidal environment. It appears that the small morphology found at Tasmanian north coast sites has likely evolved in response to the distinct environmental conditions of high periods of emersion and thermal variability in that region. The absence of plasticity in that morph and evidence for local adaptation of both juveniles and embryos is consistent with that conclusion. Likewise, genetic variation in *Hormosira* demonstrated that southeastern Australian populations are not panmictic and current boundaries and break points indicate low gene flow in *Hormosira*. The combination of field surveys, field experiments and molecular analyses provided a valuable tool to test the effect of ecologically relevant selection pressures and assess the evolutionary potential of *Hormosira*. The observed phenotype-performance gradients between habitats and even between populations suggest that different populations may have different strategies to respond to environmental change (e.g. Araújo et al. 2011) which can affect our understanding of population resilience. As a result, locally adapted populations may be at a greater risk of extinction under continuous environmental change (Eads et al. 2012). Thus, results from this thesis emphasise that spatially divergent selection regimes can shape distinct phenotypes and facilitate highly structured and specialised populations in *Hormosira*. Understanding the consequences of such population

structure is important for evaluating the species' stability and resilience, and to manage ecosystem functioning in one of the most important intertidal habitat-forming organisms in temperate Australia.

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Appendix

Table S1: Geographical coordinates, wave exposure (based on Baardseth Index [BI] and wave height [Height]), tidal conditions (mean spring tidal height [Spring], mean neap tidal height [Neap], the difference between mean spring and neap tidal height [Diff], tidal regime [Reg.]), air temperature (mean maximum and minimum for summer, winter and annual) and mean sea surface temperature from 1997 to 2009 (SST) at each site. Abbreviations for sites as in Figure 1.

Site	Latitude	Longitude	Wave		Tide				Temperature						
			BI	Height [m]	Height [m]			Reg.	Mean air [˚C]						SST [˚C]
					Spring	Neap	Diff.		Summer		Winter		Annual		
									Max	Min	Max	Min	Max	Min	
RC	40˚54’25” S	145˚32’44” E	5.0	1.0	2.6	2.0	0.6	0.21	21.50	11.45	13.35	5.00	17.30	7.30	15.00
LH	41˚03’36” S	146˚48’02” E	7.0	1.0	2.4	2.0	0.4	0.22	20.40	14.45	12.75	7.85	16.50	10.60	15.25
PP	40˚46’39” S	147˚56’37” E	13.0	1.5	1.2	1.0	0.2	0.24	20.60	14.90	13.30	9.05	16.9	11.50	15.25
BF	41˚12’48” S	148˚16’53” E	5.0	1.0	1.3	0.3	1.0	0.56	22.20	14.15	14.35	8.00	18.2	10.50	15.50
FM	41˚33’25” S	148˚17’32” E	12.0	1.5	1.2	0.0	1.2	0.81	21.55	13.25	14.35	6.15	17.8	9.10	15.25
SB	42˚33’59” S	147˚53’15” E	0.0	0.0	1.1	0.1	1.0	0.70	20.75	11.85	13.35	5.05	17.0	7.60	15.50
EN	43˚00’32” S	147˚55’58” E	6.0	1.5	1.1	0.0	1.1	0.93	16.85	11.25	11.00	6.65	13.9	8.80	14.25
AL	43˚18’48” S	147˚14’31” E	4.0	0.5	1.3	0.3	1.0	1.80	18.70	11.75	12.55	6.90	15.7	9.10	14.00
SP	43˚25’57” S	146˚58’32” E	0.0	0.0	1.3	0.3	1.0	2.02	19.80	10.10	13.00	4.85	16.3	6.90	14.25
TH	41˚55’56” S	145˚10’23” E	16.0	3.5	0.9	0.5	0.4	1.56	19.90	11.20	13.20	6.20	16.6	8.20	14.25
TE	41˚13’57” S	144˚41’15” E	4.0	0.5	0.9	0.4	0.5	1.32	19.35	12.35	12.95	7.65	16.1	9.60	14.75
MW	40˚54’27” S	144˚40’37” E	9.0	3.0	0.9	0.4	0.5	1.32	19.35	12.35	12.95	7.65	16.1	9.60	14.75